Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

In-Cell Covalent Labeling of Reactive His-Tag Fused Proteins

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Contents:

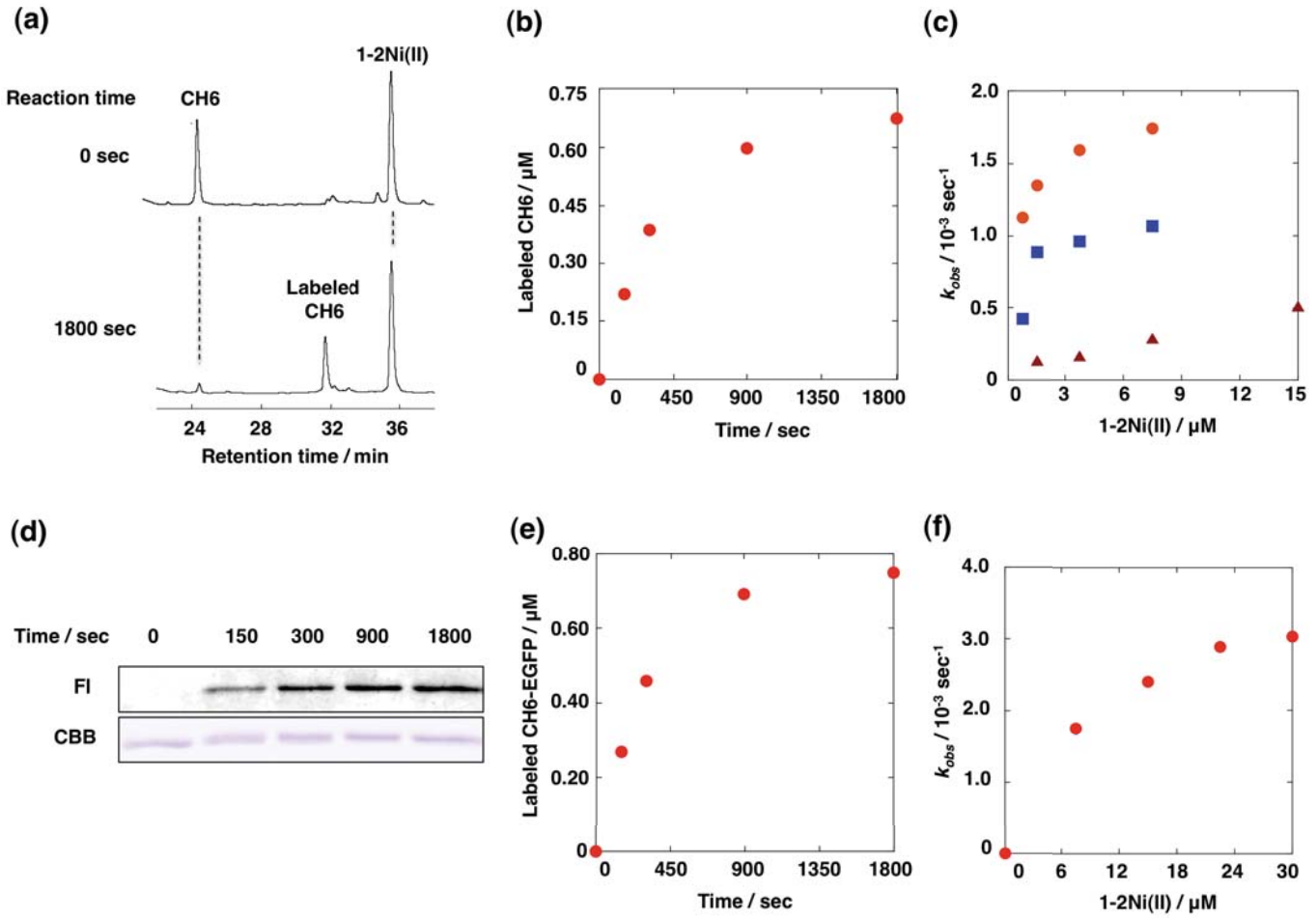


Fig S1. Kinetics analysis of the reaction between **1-2Ni(II)** and CH*n* peptides (*n* = 3, 6, 10) or CH6 tag-fused EGFP. (a) HPLC analysis of the labeling reaction of 0.75 μM CH6 peptide with 7.5 μM **1-2Ni(II)**. (b) Time-course plot of the labeling reaction between 0.75 μM CH6 peptide and 7.5 μM **1-2Ni(II)** shown in (a). (c) Plots of pseudo-first-order constants k_{obs} (sec^{-1}) of the reaction between **1-2Ni(II)** and CH3 (▲), CH6 (●) and CH10 (■) peptides against the concentration of **1-2Ni(II)**. (d) In-gel fluorescence analysis of the reaction between 0.75 μM CH6-EGFP and 30 μM **1-2Ni(II)**. (e) Time-course plot of the reaction between 0.75 μM CH6-EGFP and 30 μM **1-2Ni(II)** shown in (e). (f) Plot of k_{obs} (sec^{-1}) of the reaction between CH6-EGFP and **1-2Ni(II)** against the concentration of **1-2Ni(II)**.

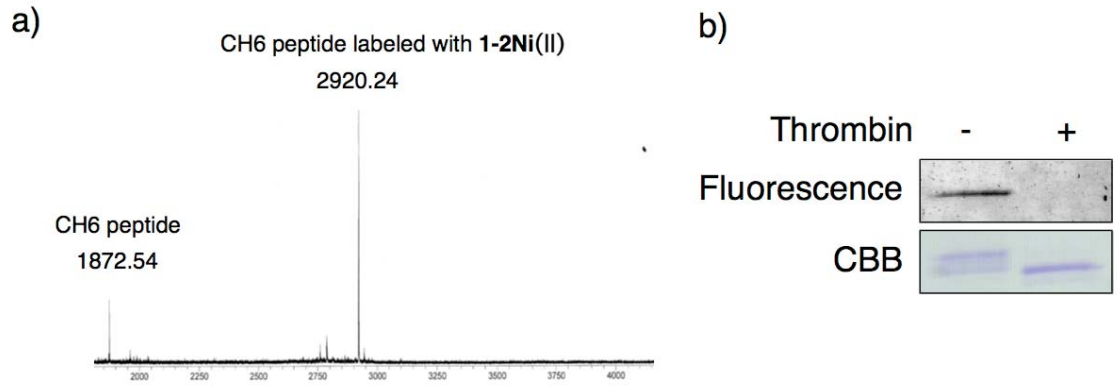


Fig S2. Identification of the labeling site of CH6-EGFP. (a) MALDI-TOF mass analysis of the peptide fragment cleaved by the thrombin digestion. CH6 peptide; GSSCHHHHHHSSGLVPR, MALDI-TOF mass calcd for $C_{77}H_{115}N_{32}O_{22}S$ $[M+H]^+$ = 1871.86; obsd 1872.54. Labeled CH6 peptide with **1-2Ni(II)** ; MALDI-TOF mass calcd for $C_{124}H_{181}N_{40}O_{41}S$ $[M+H]^+$ = 2918.30; obsd 2920.24. (b) In-gel fluorescence analysis of the labeled or unlabeled CH6-EGFP digested by thrombin. The digestion was carried out using 5 unit of thrombin (50 mM HEPES, 100 mM NaCl, pH 7.2, 22 °C, 20 hr).

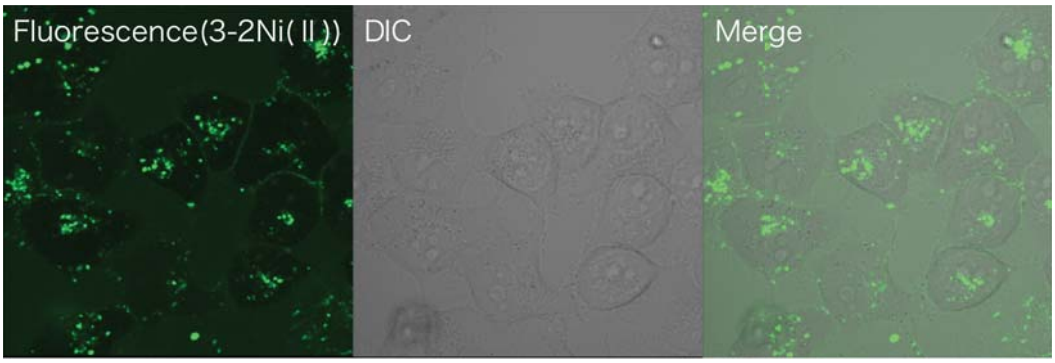


Fig S3. Fluorescence imaging of HeLa cells after the treatment of **3-2Ni(II)** and the H4-R8 carrier peptide without pyrenebutyrate. Conditions: HeLa cells (4×10^5 cells), 5 μM **3-2Ni(II)**, 5 μM H4-R8 carrier peptide without pyrenebutyrate in HBS, 30 min, 37 °C.

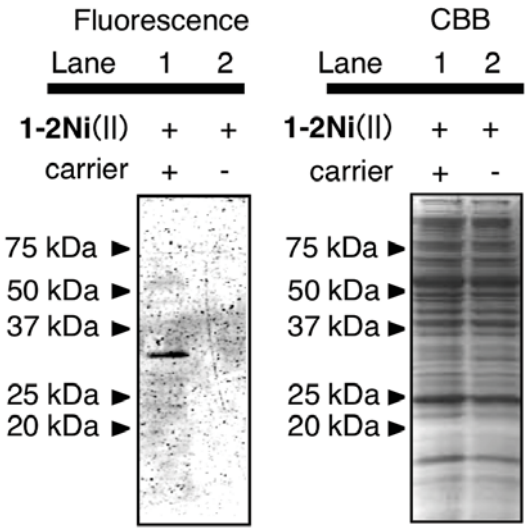


Fig S4. In-gel fluorescence (left) and CBB (right) analysis of the labeling of the CH6-EGFP with **1-2Ni(II)** inside *E. coli* cells. Conditions : 20 μM **1-2Ni(II)**, 20 μM H4-R8 carrier peptide, 50 μM pyrenebutyrate in HBS, 30 min, 37°C.

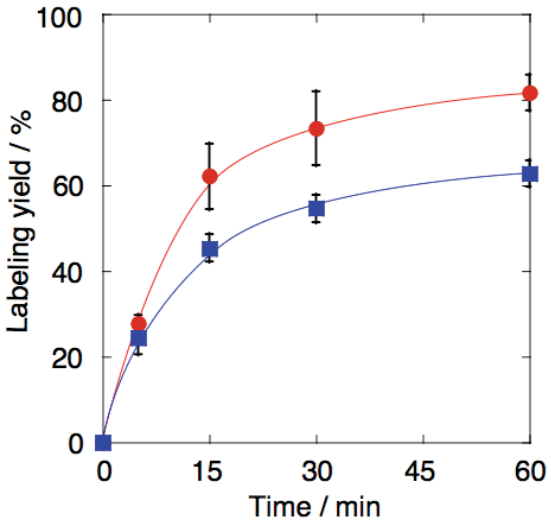


Fig S5. Time-course plot of the labeling reaction of CH6-EGFP with **1-2Ni(II)** in the absence (●) and presence (■) of H4-R8 carrier . Labeling conditions : 0.75 μM CH6-EGFP, 3.75 μM **1-2Ni(II)**, 3.75 μM H4-R8 carrier peptide, 50 mM HEPES 100 mM NaCl and 75 μM TCEP, pH 7.2, 25°C. The labeling yield was evaluated based on the band intensity observed in the in-gel fluorescence analysis, in which the band of EGFP 75% modified with 3-caroxyl-7-diethylaminocou-marine was used as a fluorescence intensity standard.

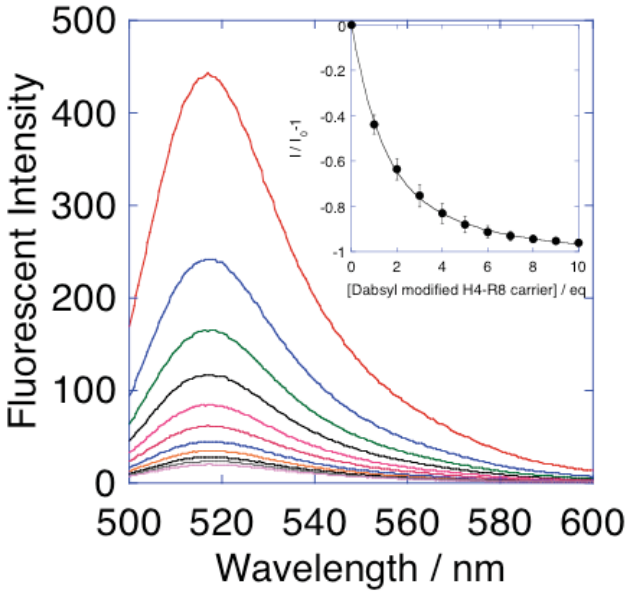


Fig S6. Evaluation of the binding affinity between **3-2Ni(II)** and the dabsyl modified H4-R8 carrier peptide (Scheme S7) by fluorescence quenching titration. Inset: curve-fitting analysis of the fluorescence emission change at 517 nm. Measurement conditions: 0.2 μM **3-2Ni(II)**, 0-2.0 μM dabsyl modified H4-R8 carrier peptide, PBS, pH 7.4, 25°C.

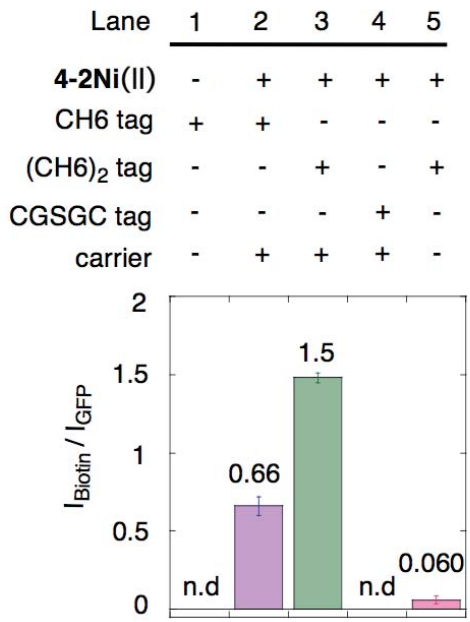


Fig S7. Comparison of the in-cell labeling efficiency of the various tags fused to EGFP-f. Y-axis indicates the relative labeling efficiency, which is defined by the relative band intensity between the biotin blotting (I_{biotin}) and the western blotting (I_{GFP}) shown Fig 3b. n. d means not detected.

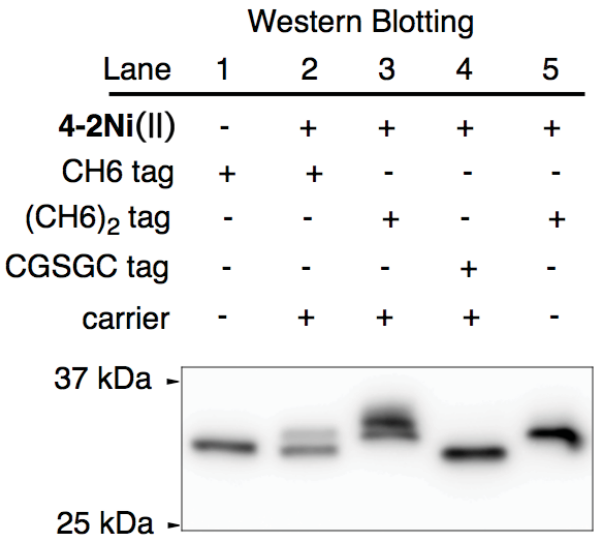


Fig S8. Magnified image of Fig 3b in the range from 25 kDa to 37 kDa. In lane 3, the new bands corresponding to the labeled protein were detected at the upper position of the original EGFP-$(CH6)_2$-f (lane 4). The labeling yield of EGFP-$(CH6)_2$-f was estimated to be 64 ± 5 % by comparing the band intensities between the labeled and the unlabeled proteins.

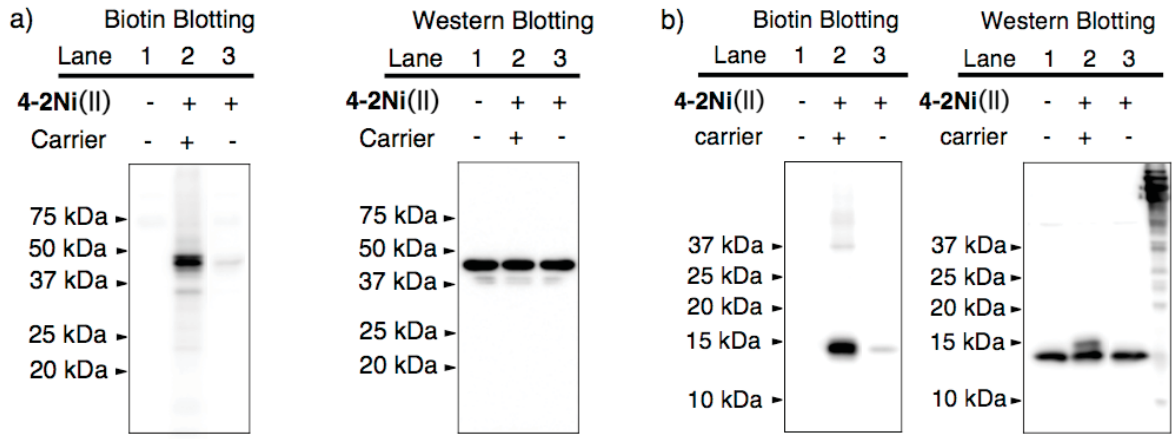


Fig S9. Blotting analysis of the in-cell labeling of the $(CH6)_2$ tag fused FKBP12-EFYP (a) and FRB (b). The biotin blotting was conducted using the streptavidin-HRP conjugate and the western blotting was conducted using anti-FKBP12 antibody (a) and anti-6xHis antibody (b). Conditions: HeLa cells (4×10^5 cells), 5 μM **4-2Ni(II)**, 5 μM H4-R8 carrier peptide, 50 μM pyrenebutyrate in HBS, 30 min, 37 °C.

EXPERIMETAL DETAILS

Kinetics Analysis of the Reaction of the CHn peptides (n = 3, 6 and 10) with 1-2Ni(II)

The reaction of **1-2Ni(II)** (0.75~15 μM) with the CHn peptide (n = 3, 6 or 10) (0.75 μM) was carried out in a degassed buffer solution (50 mM HEPES, 100 mM NaCl, 75 μM TCEP, pH 7.2) at 25 °C. The reaction was quenched by addition of TFA (2 wt% in final concentration) at appropriate times. The sampling solution was subjected to HPLC analysis, in which the decrease of the peak of the starting peptide was detected by UV absorbance at 220 nm to calculate the concentration of the labeled peptide (Figure S1b). Each k_{obs} (sec^{-1}) value was calculated using the equation of $k_{obs} = \ln 2/t_{1/2}$, wherein $t_{1/2}$ is the reaction half-time for the formation of the labeled peptide. According to Michaelis-Menten enzyme kinetics, the second-order constant k_2 values ($M^{-1}sec^{-1}$) of CH6 and CH10 peptide were calculated based on the equation of $k_2 = k_{obs,max} / K_m$, wherein $k_{obs,max}$ is the maximum value of k_{obs} observed in the saturation curve in Figure S1c, and K_m is defined as the concentration of **1-2Ni(II)** that gives the half value of $k_{obs,max}$. In the case of CH3 peptide, k_2 was calculated based on the equation of $k_{obs} = k_2$[**1-2Ni(II)**].

Covalent Labeling of the CH6-EGFP

A solution of CH6-EGFP (0.75 μM in 50 mM HEPES, 100 mM NaCl, 75 μM TCEP, pH 7.2) was mixed with **1-2Ni(II)**, **1** or **2-Ni(II)** (3.75 μM in final concentration). The mixture was incubated at 25°C for 60 min, in which time 40 μL of the mixture was sampled at the appropriate point and treated with 2×Laemmli buffer to quench the labeling reaction. The sampling solutions were heated at 95°C for 3 min and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The in-gel fluorescence analysis was performed with LAS-4000 lumino image analyzer (GE Healthcare) by EPI mode (365 nm excitation, L41 filter). The labeling yield (%) was evaluated based on the fluorescence band intensity of the labeled protein, in which the band of EGFP 75% modified with 3-caroxyl-7-diethylaminocoumarine was used as a fluorescence intensity standard.

Kinetics Analysis of the Labeling Reaction of CH6-EGFP with the 1-2Ni(II)

A solution of CH6-EGFP (0.75 μM in 50 mM HEPES, 100 mM NaCl, 75 μM TCEP, pH 7.2) was mixed with **1-2Ni(II)** (5.62~22.5 μM in final concentration). The mixture was incubated at 25°C for 30 min, in which time 30 μL of the mixture was sampled at the appropriate point and treated with TFA (final conc 0.6w%) to quench the labeling reaction. The solutions were then neutralized with 10 μL of 1M Tris-HCl buffer containing 50 mM EDTA, mixed 10 μL of 5×Laemmli buffer, heated at 95°C for 3 min and subjected to SDS-PAGE. The kinetics analysis was done by the same method as described in the CHn peptide labeling.

Identification of Labeling Site of CH6-EGFP

CH6-EGFP (20 μg) was mixed with **1-2Ni(II)** and incubated at 25°C. After 60 min, the labeled CH6-EGFP was purified by size exclusion chromatography (PD-10 column, GE Healthcare) and treated with thrombin (5 unit in 50 mM HEPES, 100 mM NaCl, pH 7.2, 22 °C, 20 hr) to cleave the CH_6-tag fragment. The digested peptide sample was condensed with Zip-tip C18 (Millipore) and analyzed by MALDI-TOF mass. The digested EGFP sample were mixed with 2×Laemmli buffer, heated at 95°C for 3 min, and subjected to SDS-PAGE. The in-gel fluorescence analysis was performed with LAS-4000 lumino image analyzer (GE Healthcare) by EPI mode (365 nm excitation, L41 filter).

Cell Culture and Recombinant Protein Expression in HeLa and A549 Cells

HeLa and A549 cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 μg/mL), and amphotericin B (250 ng/mL). Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. A subculture was performed every 3-4 days from subconfluent (<80%) cultures using a trypsin-EDTA solution. Transfection of cDNA plasmids (1 μg) was carried out in a 35 mm dish (falcon) using plus reagent (1 μL) and Lipofectamine LTX (2 μL). The cells were subjected to labeling experiments after 18-20 h of the transfection.

Fluorescence Imaging of introduction 3-2Ni(II) into HeLa cells

The native HeLa cells (~4×10^5 cells) were washed twice with HEPES-buffered saline (HBS, containing 107 mM NaCl, 6 mM KCl, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 11.5 mM glucose, 20 mM HEPES, adjusted to pH 7.4 with NaOH). The cells were first treated with pyrenebutyrate (1-pyrenebutyric, 67 μM) in HBS for 5 min at RT, and then the mixture of **3-2Ni(II)** (5 μM , final conc.) and H4-R8 carrier (5 μM, final conc.) in HBS was added, at which the final concentration of pyrenebutyrate was 50 μM. After incubation for 30 min at 37°C, the cells were washed twice with HBS. The cells were analyzed using a confocal laser scanning microscope (CLSM; Olympus, FLUOVIEW FV1000).

Covalent Labeling of the CH6-EGFP in *E. coli*

The pET 28a plasmid coding CH6-EGFP was transformed into *E. Coli* BL21(DE3)pLysS. The *E. Coli* cells were grown in LB medium (5 mL) at 37°C overnight and collected by centrifugation (5,000 rpm, 5 min). The cells washed twice with HBS. The cells were first treated with pyrenebutyrate (67 μM) in HBS for 5 min at RT, and then the mixture of **1-2Ni(II)** (20 μM, final conc.) and H4-R8 carrier (20 μM, final conc.) in HBS was added, at which the final concentration of pyrenebutyrate was 50 μM. After incubation for 30 min at 37°C, the cells were washed twice with HBS containing 10 mM EDTA and lysed by sonication in HBS containing 10 mM EDTA. Insoluble materials were removed by centrifugation (12,000 rpm, 10 min, x2) to collect the soluble fraction. The supernatants were mixed with 2×Laemmli buffer and heated at 95°C for 3 min and subjected to SDS-PAGE. The in-gel fluorescence analysis was performed with LAS-4000 lumino image analyzer (GE Healthcare) by EPI mode (365 nm excitation, L41 filter).

General Method of In-Cell Protein Labeling

The HeLa cells expressing a tag-fused protein (~ 4×10^5 cells) were washed twice with HBS. The cells were first treated with pyrenebutyrate (67 μM) in HBS for 5 min at RT, and then the mixture of **4-2Ni(II)** (5 μM , final conc.) and H4-R8 carrier (5 μM, final conc.) in HBS was added, at which the final concentration of pyrenebutyrate was 50 μM. After incubation for 30 min at 37°C, the cells were washed twice with HBS and lysed with 100 μL of RIPA lysis buffer (pH 7.6 25 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 1% Deoxycholic acid) containing 1% protease inhibitor cocktail set III (Novagen) and 10 mM EDTA on ice for 15 min. The lysed cells were collected by cell scraper and the insoluble material was removed by centrifugation at 12,000 rpm for 10 min. The supernatants were mixed with 2×Laemmli buffer and heated at 95 °C for 3 min. The lysates were subjected to SDS-PAGE and then transferred onto an Immun-Blot PVDF membrane (Bio-Rad). The membranes were blocked in TBS-T buffer (50 mM Tris-HCl, 150 mM NaCl, 0.05 % tween 20) containing 5 % skimmed milk for 1 h at RT. The immunodetections of the tag-fused EGFP-f, FKBP12-EYFP and FRB were performed with rabbit polyclonal antibody against GFP (1 : 2000 dilution, abcam), rabbit polyclonal antibody against FKBP12 (1 : 2000 dilution, abcam), and rabbit polyclonal antibody against 6×His (1 : 2000 dilution, abcam) as primary antibody, respectively, and goat anti-rabbit horseradish peroxidase (HRP)-conjugated IgG antibody (1:5000 dilution, Santa Cruz Biotechnology) as a secondary antibody. The biotin blotting was performed with streptavidin-HRP conjugated (1: 5000 dilution; Invitrogen). The chemiluminescence analyses were performed by ImageQuant LAS-4000 (GE Healthcare) using Chemi-Lumi One (Nacalai Tesque) reagent.

Photo-Cross-Linking of the Intracellular Proteins

A549 cells (~ 4×10^5 cells) were co-transfected with pFRB-$(CH6)_2$ plasmid and pFKBP12-EYFP plasmid using Lipofectamine LTX (Invitrogen) by following the standard protocol. After incubation of the cells for 18 h at 37 °C in a CO_2 incubator, the medium was replaced with DMEM in the presence or absence of rapamycin (5 μM), and the cells were incubated at 37°C for 20 min. The labeling reaction was performed with **5-2Ni(II)** (5 μM) at 4 °C for 30 min by following the labeling procedure describe above. The cells were then exposed to 365 nm light by using a UV lamp (8 W) at 4 cm distance from the top of the cell culture dish (without the lid) on ice for 20 min. The cells were washed twice with PBS and lysed using RIPA lysis buffer containing 10 mM EDTA. The samples were analyzed by Western blotting using anti GFP-antibody and anti-6×His antibody. The chemiluminescence signal was detected using ECL Prime Western Blotting Detection Reagent (GE Healthcare) by ImageQuant LAS-4000 (GE Healthcare).

Florescence Titration with the Dabsyl Modified H4-R8 Carrier Peptide

A PBS solution of **3-2Ni(II)** (0.2 μM) in a quartz cell was titrated with a solution of the dabsyl modified H4-R8 carrier at 25°C. The concentration of the titrant solution of dabsyl modified H4-R8 carrier peptide was determined based on the reported extinction coefficient of dabsyl (ε_{420}= 33,000 $M^{-1}\cdot cm^{-1}$).[S1] The fluorescence spectra were measured using PerkinElmer LS55 spectrofluorophotometer (λ_{ex} = 480 nm). The plot of the fluorescence intensities at 517 nm were

analyzed using nonlinear least-square curve-fitting assuming 1:1 binding to evaluate the apparent binding constant (K_d, M).

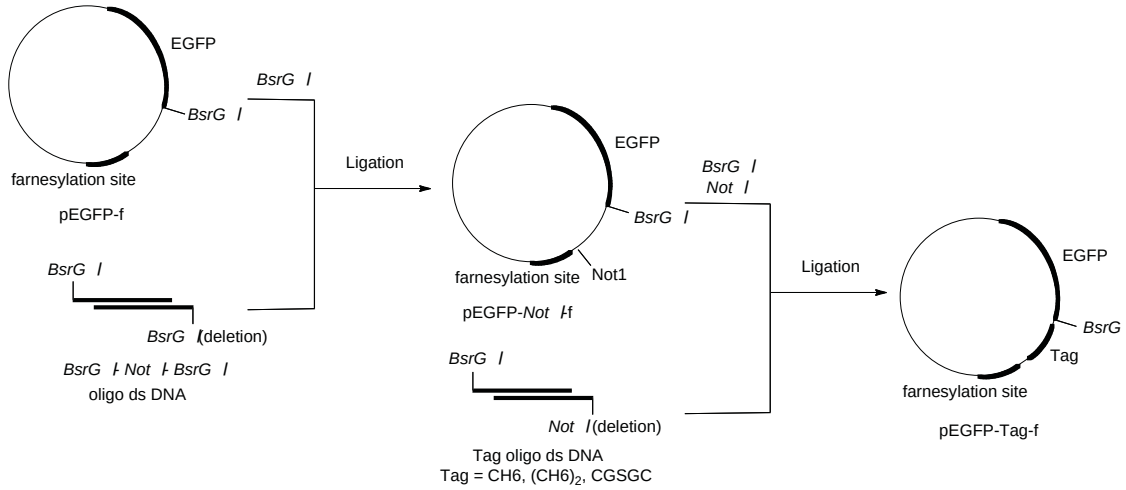
Construction of CH6-EGFP Plasmid

The oligo DNA fragments coding CH6 tag and thrombin cleavage site (MGSSCHHHHHHSGLVPRGS) was inserted into *Nco1-Nde1* site of pET-28a plasmid, which codes EGFP at *Not1-EcoR1* site.[S2] The sequences of the 5'-phosphorylated DNA fragments were as follows: 5'-cat ggg cag cag ctg cca tca tca tca tca tca cag cag cgg cct ggt gcc gcg cgg cag cgg-3' (forward) and 5'- ata ccg ctg ccg cgc ggc acc agg ccg ctg ctg tga tga tga tga tga tgg cag ctg ctg cc-3'(backward).

Expression of CH6-Tag Fused EGFP

pET-28a plasmid coding CH6-EGFP was transformed into *E. coli*. BL21(DE3) pLysS. The cells were grown in 500 mL of LB medium at 37°C until an optical density (OD) at 600 nm increased to 0.6 and further grown at 16°C for 24h with IPTG induction (0.1 mM). The cells were spun down for 10 min at 4000 rpm. The cells were re-suspended in 40 mL of lysis buffer (50 mM HEPES 100 mM NaCl, pH 7.2, 10% glycerol) and lysed by sonication (10 shots x 20, Branson Sonifier 450). Insoluble materials were removed by centrifugation for 10 min at 12,000 rpm (x2) to collect the soluble fraction containing CH6-EGFP. Purification was performed with TALON resin (Clontech). The soluble fraction (50 mL) was adsorbed on 2 mL of TALON resin in a plastic column and then washed with HEPES buffer (50mM HEPES, 100 mM NaCl, pH 7.2) containing 5 mM imidazole. The resin-bound protein was eluted from the column with HEPES buffer containing 150 mM imidazole. The fractions containing of the purified EGFP (confirmed by SDS-PAGE) was dialyzed twice with HEPES buffer to remove the excess imidazole. The concentration of CH6-EGFP was determined based on UV absorbance at 489 nm using the reported extinction coefficient of EGFP (ε_{489} = 55,000 $M^{-1}\cdot cm^{-1}$).[S3]

Construction of EGFP-CH6-f, EGFP-$(CH6)_2$-f and EGFP-CGSGC-f Plasmids



The oligo DNA fragments coding *Not1* site sequence were inserted into *BsrG1* site of pEGFP-f plasmid (Clontech). The sequences of the 5'-phosphorylated DNA fragments were as follows: 5'- GTA CAA GGT AGC GGC GCG GCG CCG C-3' (forward) and 5'- GTA CGC GGC GCC GCG CCG CTA CCC TT-3' (backward). The oligo DNA fragments coding CH6 tag (CHHHHHH), $(CH6)_2$ tag (CHHHHHHGSGCHHHHHH) or CGSGC tag (CGSGC) were inserted into *BsrG1*-*Not1* site of pEGFP-*Not1*-f. The sequences of the 5'-phosphorylated DNA fragments were as follows:

CH6 tag : 5'- GTA CAA GGG TAG CGG CTG CCA TCA CCA TCA CCA TCA CGG-3' (forward) and 5'-GGC CCC GTG ATG GTG ATG GTG ATG GCA GCC GCT ACC CTT-3' (backward).

$(CH6)_2$ tag : 5'- GTA CAA GGG TAG CGG CTG CCA TCA CCA TCA CCA TCA CGG TAG CGG CTG CCA TCA CCA TCA CCA TCA CGG-3' (forward) and 5'-GGC CCC GTG ATG GTG ATG GTG ATG GCA GCC GCT ACC GTG ATG GTG ATG GTG ATG GCA GCC GCT ACC CTT-3'(backward).

CGSGC tag : 5'-GTA CAA GGG TAG CGG CTG CGG CAG CGG TTG CGG-3', (forward) and 5'- GGC CCC GCA ACC GCT GCC GCA GCC GCT ACC CTT-3'(backward).

The pEGFP-Tag-f plasmids were purified by using Qiagen Plasmid Maxi kit (Qiagen) for transfection into mammalian cells.

Construction of $(CH6)_2$ -FKBP12-EYFP Plasmid

The oligo DNA fragments coding $(CH6)_2$ tag was inserted into *Xho1*-*Hind*Ⅲ site of pEYFP-N1 plasmid coding FKBP12-EYFP hybrid. The sequences of the 5'-phosphorylated DNA fragment were as follows: $(CH6)_2$ tag : 5'- TCG AGA TGG GTA GCG GCT GCC ATC ACC ATC ACC ATC ACG GTA GCG GCT GCC ATC ACC ATC ACC ATC ACA-3' (forward) and 5'- AGC TGT GAT GGT GAT GGT GAT GGC AGC CGC TAC CGT GAT GGT GAT GGT GAT GGC AGC CGC TAC CCA TC-3'(backward).

The $(CH6)_2$-FKBP12-EYFP plasmid was purified by using Qiagen Plasmid Maxi kit (Qiagen) for transfection into mammalian cells.

Construction of FRB-$(CH6)_2$ Plasmid

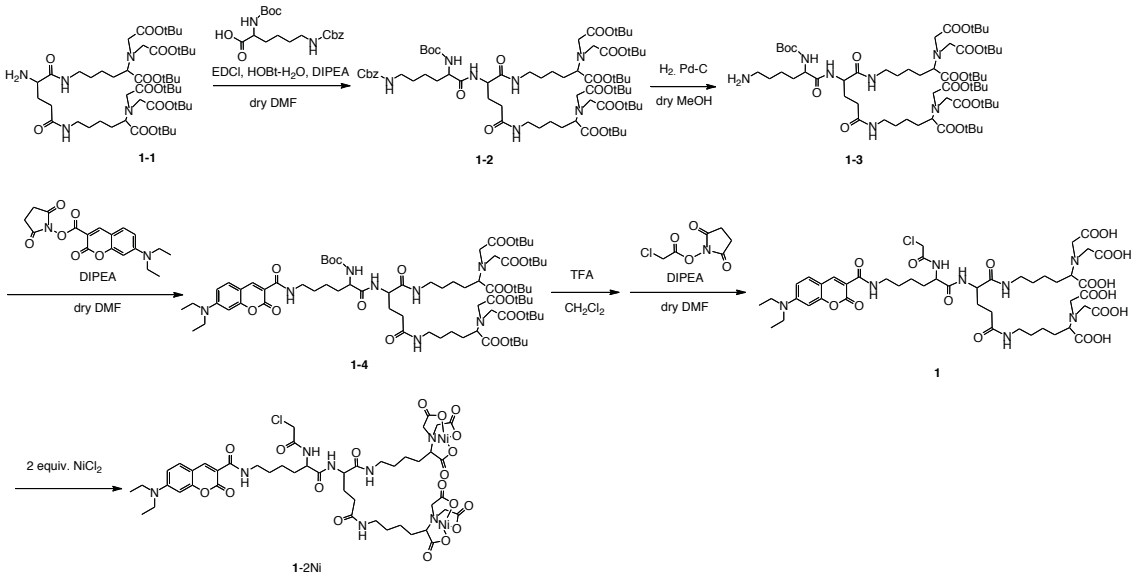
The oligo DNA fragments coding $(CH6)_2$ tag was inserted into *Spe1*- *BamH1* site of pC_4-R_HE plasmid(ARIAD Pharmaceuticals) coding FRB protein. The sequences of the 5'-phosphorylated DNA fragment were as follows: $(CH6)_2$ tag : 5'-CTA GTG GCG GTT GCC ATC ACC ATC ACC ATC ACG GTA GCG GCT GCC ATC ACC ATC ACC ATC ACT-3' (forward) and 5'- GAT CAG TGA TGG TGA TGG TGA TGG CAG CCG CTA CCG TGA TGG TGA TGG TGA TGG CAA CCG CCA-3' (backward).

The FRB-$(CH6)_2$ plasmid was purified by using Qiagen Plasmid Maxi kit (Qiagen) for transfection into mammalian cells.

SYNTHESIS AND CHARACTEIZATION OF THE COMPOUNDS

General Methods. Unless otherwise noted, all chemical reagents were purchased from commercial suppliers (Sigma-Aldrich, Tokyo Chemical Industry, Wako Pure Chemical Industries, Acros Organics, Thermo Scientific, Life Technologies, Sasaki Chemical, or Watanabe Chemical Industries) and used without further purification. ^{1}H-NMR spectra were recorded using a Varian Mercury 400 (400 MHz) spectrometer, and the chemical shifts (δ, ppm) are referenced to the respective solvent. ^{13}C-NMR spectra were recorded using a JEOL JNM-ECA 600 (600 MHz) spectrometer, and the chemical shifts (δ, ppm) are referenced to the respective solvent.High-resolution electrospray ionization quadrupole Fourier transform mass spectrometry (HR-ESIMS) spectra were performed on a Bruker apex-ultra (7T) mass spectrometer. MALDI was recorded using autoflex II (Bruker Daltonics). Reverse-phase HPLC was conducted with a Lachrom (Hitachi) instrument with C18 columns.

Synthesis of **1-2Ni(II)**



Scheme S1. Synthesis of **1-2Ni(II)**.

Compound 1-2

To a stirred solution of **1-1**[S4] (154 mg, 0.16 mmol) in dry DMF (5 mL) were added Boc-Lys(Z)-OH (70 mg, 0.19 mmol), EDCl (44 mg, 0.23 mmol), HOBt-H_2O (35 mg, 0.23 mmol) and DIPEA (107 μL, 0.62 mmol). The reaction mixture was stirred for 12 h at RT. After removal of solvent in vacuo, the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with saturated $NaHCO_3$ aq. (50 mL x2) and brine (50 mL) followed by drying over anhydrous Na_2SO_4. The solvent was removed in vacuo to give **1-2** (199 mg, 97%) as a brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.27~7.36 (m, 5H), 5.10 (s, 1H), 4.33~4.39 (m, 1H), 3.97~4.03 (m, 1H), 3.41~3.47 (m, 8H), 3.26~3.29 (m, 2H), 2.37~2.46 (m, 1H), 2.21~2.30 (m, 1H), 2.00~2.17 (m, 2H), 1.51~1.67 (m, 14H), 1.42~1.46(m, 67H). HR-ESI-MS calcd for $C_{68}H_{116}N_7O_{19}$ $[M+H]^+$ = 1334.8321; obsd 1334.8310

Compound 1-3

A mixture of **1-2** (50 mg, 38 μmol) and 10 wt% Pd-C (5 mg) in dry MeOH (5 mL) was stirred for 4 h at RT under H_2 atmosphere. After removal of Pd-C by filtration, the filtrate was concentrated in vacuo to give **1-3** (45 mg, 99%) as a brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.12~4.34 (m, 2H), 3.36~3.51 (m, 8H), 3.15~3.32 (m, 6H), 2.94~3.07 (m, 2H), 2.38~2.47 (m, 1H), 2.13~2.31 (m, 1H), 2.06~2.17 (m, 2H), 1.71~1.87(m, 4H), 1.51~1.65 (m, 10H), 1.43~1.46 (m, 67H), ^{13}C-NMR (600 MHz, CD_3OD) δ 174.91, 174.82, 173.74, 173.70, 173,41, 172.49, 172.40, 172.39, 158.07, 82.49, 82.47, 82.10, 82.07, 80.80, 79.49, 66.68, 56.06, 54.84, 54.81, 54.31, 40.53, 40.38, 40.35, 33.12, 32.37, 31.48, 31.36, 30.08, 30.04, 29.10, 28.80, 28.53, 28.52, 28.44, 28.08, 24.51, 24.43, 23.59. HR-ESI-MS calcd for $C_{60}H_{110}N_7O_{17}$ $[M+H]^+$ = 1200.7953; obsd 1200.7929

Compound 1-4

To a stirred solution of **1-3** (45 mg, 37 μmol) in dry DMF (3 mL) were added 7-diethylaminocoumarin-3-carboxylic acid *N*-hydroxysuccinimide ester[S5] (15 mg, 41 μmol) and DIPEA (7.8 μL, 45 μmol). The reaction mixture was stirred for 3 h at RT. After concentration in vacuo, the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with saturated $NaHCO_3$ aq. (50 mL x2) and brine (50 mL) followed by drying over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by column chlomatography on SiO_2 ($CHCl_3$: MeOH = 40 : 1) to give **1-4** (21 mg, 39%) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.86 (t, *J* = 6.4 Hz, 1H), 8.71(s, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.42 (d, *J* = 9.2 Hz, 1H), 7,27 (t, *J* = 7.2 Hz, 1H), 6.65 (d, *J* = 9.2 Hz, 1H), 6.60(t, *J* = 7.2 Hz, 1H), 6.50 (s, 1H), 5.54 (d, *J* = 7.2 Hz, 1H), 4.35 (dt, *J* = 6.0, 6.0 Hz, 1H), 3.97~4.00 (m, 1H), 3.40~3.71 (m, 14H), 3.16~3.38 (m, 6H), 2.25~2.40 (m, 2H), 1.81~1.95 (m, 2H), 1.51~1.75 (m, 14H), 1.41~1.45 (m, 67H), 1.24 (t, *J* = 7.2 Hz, 6H). ^{13}C-NMR (600 MHz, $CDCl_3$): δ 173.24, 172.52, 172.44, 172.35, 171.02, 170.71, 163.51, 162.76, 157.65, 156.41, 152.54, 148.14, 131.11, 110.29, 109.96, 108.42, 96.58, 81.15, 80.98, 80.82, 80.61, 79.93, 65.35, 64.87, 55.70, 53.98, 53.69, 53.23, 45.08, 39.57, 39.42, 38.60, 32.50, 31.05, 30.37, 29.71, 29.46, 29.16, 28.38, 28.23, 28.22, 28.18, 28.15, 28.13, 27.69, 23.49, 22.85, 22.66, 12.43. HR-ESI-MS calcd for $C_{74}H_{123}N_8O_{20}$ $[M+H]^+$ = 1443.8880; obsd 1443.8840

Compound 1

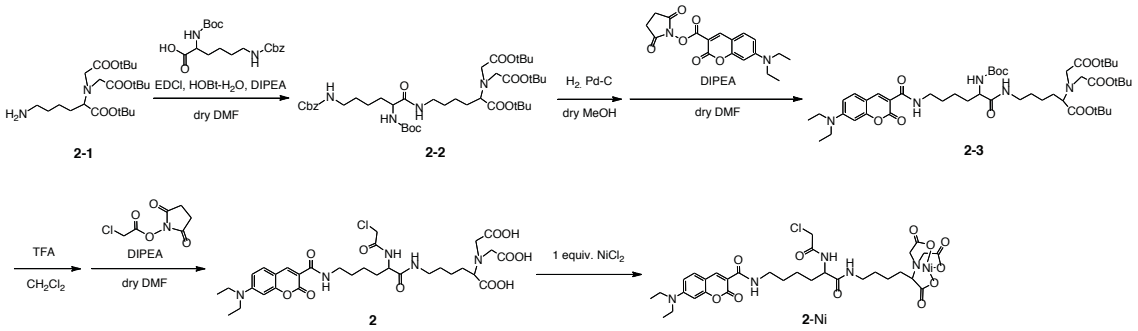
To a stirred solution of **1-4** (21 mg, 14 μmol) in dry CH_2Cl_2 (1.5 mL) was added dropwise TFA (1.0 mL) and the mixture was stirred for 12h at RT. After removal of the solvent in vacuo, the residual TFA was azeotropically removed with toluene (1 mL) to give the crude deprotected product. To a solution of the crude product in dry DMF (1 mL) was added chloroacetic acid *N*-hydroxysuccinimide ester[S6] (2.2 mg, 11 μmol) and DIEA (9.0 μL, 69 μmol). The reaction mixture was stirred for 2 h at RT. After removal of the solvent in vacuo, the residue was purified by reverse-phase HPLC. The subsequent lyphilization afforded **1** (4.5 mg, 30% in 2 steps from **1-3**) as a yellow solid. HPLC conditions: column; YMC triart-C18, 250 x 5 mm, mobile phase; CH_3CN (containing 0.1% TFA) / H_2O (containing 0.1% TFA) = 20 / 80 ~ 60 / 40 (linear gradient over 40 min), flow rate : 3 mL / min, detection UV wavelength; 220 nm. The purity of

1 was confirmed to be > 95% by HPLC analysis. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.62~8.64 (m, 2H), 8.33 (d, *J* = 7.2Hz, 1H), 8.14 (d, *J* = 7.2, 1H), 7.74~7.80 (m, 2H), 7.67 (d, *J* = 8.8 Hz, 1H), 6.81 (d, *J* = 8.8, 1H), 6.61 (s, 1H), 4.23~4.35 (m, 1H), 4.07~4.19 (m, 3H), 3.44~3.53 (m, 14H), 2.97~3.09 (m, 6H), 2.05~2.09 (m, 2H), 1.79~1.90 (m, 2H), 1.55~1.67 (m, 8H), 1.36~1.42 (m, 8H), 1.14 (t, *J* = 6.8 Hz, 6H); HR-ESI-MS calcd for $C_{47}H_{67}ClN_8O_{10}Na$ $[M+Na]^+$ = 1105.4109; obsd 1105.4103.

Compound 1-2Ni(II)

A purified **1** was dissolved in DMSO (100 μL). The concentration of **1** was determined to be 1.70 mM based on UV absorbance at 431 nm using the extinction coefficient of 7-diethylaminocoumarin (ε_{431} = 42,000 $M^{-1} \cdot cm^{-1}$).[S5] An aqueous $NiCl_2$ solution (3.40 mM, 100 μL) was added to give a stock solution of **1-2Ni(II)** (840 μM).

Synthesis of **2-Ni(II)**



Scheme S2. Synthesis of **2-Ni(II)**.

Compound 2-2

To a solution of **2-1**[S4] (200 mg, 0.47 mmol) in dry DMF (5 mL) were added Boc-Lys(Z)-OH (212 mg, 0.558 mmol), EDCl (133 mg, 0.70 mmol), HOBt-H_2O (107 mg, 0.70 mmol) and DIPEA 323 μl (1.9 mmol, 4.0 eq). The reaction mixture was stirred for 12 h at RT. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with saturated $NaHCO_3$ aq. (50 mL x2) and brine (50 mL) followed by drying over anhydrous Na_2SO_4. The solvent was removed in vacuo to give **2-2** (357 mg, 97%) as a brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.31~7.35 (m, 5H), 5.08 (s, 2H), 4.05~4.09 (m, 1H), 3.48 (d, *J* = 9.4 Hz, 4H), 3.11~3.30 (m, 5H), 1.55~1.69 (m, 12H), 1.46 (s, 9H), 1.44 (s, 18H), 1.42 (s, 9H). ^{13}C-NMR (600 MHz, $CDCl_3$): δ 172.42, 172.14, 170.82, 156.61, 155.81, 136.65, 128.49, 128.11, 128.06, 81.22, 80.90, 79.70, 66.56, 64.67, 54.21, 53.78, 50.67, 40.59, 39.18, 32.53, 29.80, 29.35, 28.36, 28.34, 28.22, 28.12, 22.79, 22.51. HR-ESI-MS calcd for $C_{41}H_{69}N_4O_{11}$ $[M+H]^+$ = 793.4957; obsd 793.4932.

Compound 2-3

A solution of **2-2** (192 mg, 0.24 mmol) and 10 wt% Pd-C (20 mg) in dry MeOH (5 mL) was stirred for 4 h at RT under H_2 atmosphere. After removal of Pd-C by filtration, a filtrate was evaporated to give the crude deprotected product from **2-2**. To a stirred solution of the deprotected product in dry DMF (3 mL) were added 7-diethylaminocoumarin-3-carboxylic acid[S3] (68 mg, 0.29 mmol), EDCI (67 mg, 0.35 mmol), HOBt-H_2O (54 mg, 0.35 mmol) and DIPEA (170 μL, 0.97 mmol). The reaction mixture was stirred for 12 h at RT. After removal of the solvent in vacuo, the residue was diluted with $CHCl_3$ (50 mL). The solution was washed with sat. $NaHCO_3$ aq. (50 mL x 2) and brine (50 mL) followed by drying over anhydrous Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by column chlomatography on SiO_2 (Hexane : AcOEt = 1 : 2) to give **2-3** (69 mg, 31%) as yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.21 (t, *J* = 5.6 Hz, 1H), 8.70 (s, 1H), 8.43 (d, *J* = 8.8 Hz, 1H), 6.64 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 6.43~6.48 (m, 1H), 4.05~4.09 (m, 1H), 3.39~3.47 (m, 10H), 3.22~3.31 (m, 3H), 1.55~1.69 (m, 12H), 1.46 (s, 9H), 1.44 (s, 18H), 1.42 (s, 9H), 1.24 (t, *J* = 7.2 Hz, 6H). ^{13}C-NMR (600 MHz, $CDCl_3$): δ 172.39, 172.10, 170.74, 163.22, 162.73, 157.61, 155.76, 152.49, 148.01, 131.09, 110.39, 109.92, 108.39, 96.57, 81.08, 80.76, 79.55, 64.79, 60.39, 54.75, 45.07, 39.24, 39.08, 32.25, 29.91, 29.36, 28.61, 28.37, 28.61, 28.37, 28.23, 28.15, 22.90, 22.84, 12.43. HR-ESI-MS calcd for $C_{47}H_{76}N_5O_{12}$ $[M+H]^+$ = 902.5485; obsd 902.5456.

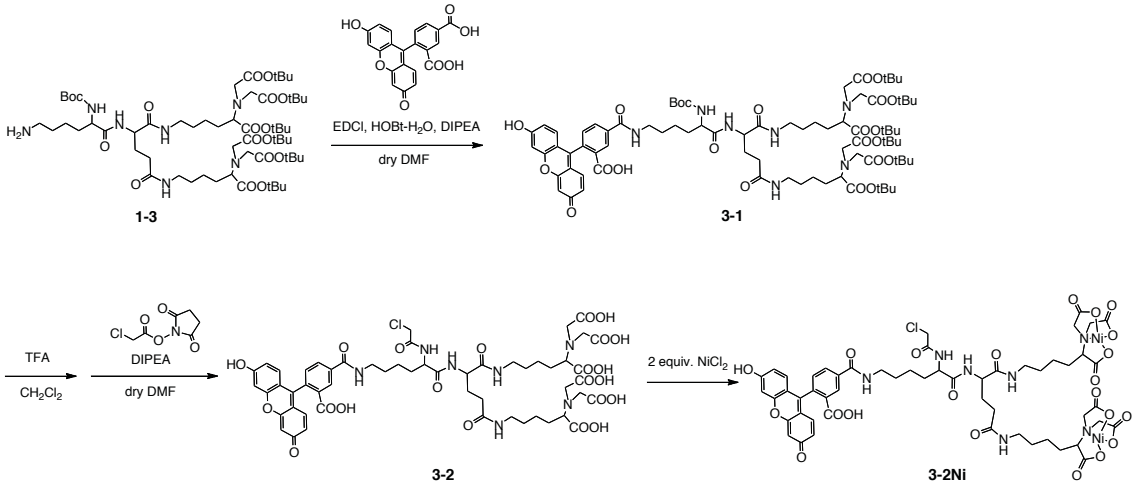
Compound 2

To a stirred solution of **2-3** (18 mg, 20 μmol) in dry CH_2Cl_2 (1.5 mL) was added dropwise TFA (1.0 mL) and the mixture was stirred for 6 h at RT. After removal of the solvent in vacuo, the residual TFA was azeotropically removed with toluene (1 mL) to give the crude deprotected porduct. To a solution of the crude product in dry DMF (2 mL) was added chloroacetic acid *N*-hydroxysuccinimide ester (3.9 mg, 20 μmol) and DIEA (21 μL, 123 μmol). The reaction mixture was stirred for 3 h at RT. After removal of the solvent in vacuo, the residue was purified by reverse-phase HPLC. The subsequent lyphilization afforded **2** (10.2 mg, 47 % in 2 steps from **2-3**) as a yellow solid. HPLC conditions: column; YMC triart-C18, 250 x 5 mm, mobile phase; CH_3CN (containing 0.1% TFA) / H_2O (containing 0.1% TFA) = 20 / 80 ~ 55 / 45 (linear gradient over 40 min), flow rate : 3 mL / min, detection UV wavelength; 220 nm. The purity of **2** was confirmed to be > 95% by HPLC analysis. ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.61~8.63 (m, 2H), 8.27 (d, *J* = 8.4 Hz, 1H), 8.01 (t, *J* = 5.6 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 6.80 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.61 (d, *J* = 2.4 Hz, 1H), 4.18~4.24 (m, 1H), 4.09 (d, *J* = 20 Hz, 2H), 3.49~3.61 (m, 10H), 2.94~3.05 (m, 4H), 1.48~1.64 (m, 6H), 1.26~1.39 (m, 6H), 1.14 (t, *J* = 6.8 Hz, 6H); HR-ESI-MS calcd for $C_{32}H_{45}ClN_5O_{11}$ $[M+H]^+$ = 710.2799; obsd 710.2789

Compound 2-Ni(II)

A purified **2** was dissolved in DMSO (100 µL). The concentration of **2** was determined to be 6.4 mM based on UV absorbance at 431 nm using the extinction coefficient of 7-diethylaminocoumarin (ε_{431} = 42,000 M^{-1} · cm^{-1}). An aqueous $NiCl_2$ solution (6.4 mM, 100 µL) was added to give a stock solution of **2-Ni(II)** (3.2 mM).

Synthesis of **3-2Ni(II)**



Scheme S3. Synthesis of **3-2Ni(II)**.

Compound 3-1

To a stirred solution of **1-3** (70 mg, 58 µmol) in dry DMF (5 mL) were added 5-carboxy fluorescein (26 mg, 70 µmol), EDCl (17 mg, 87 µmol), HOBt-H_2O (13 mg, 87 µmol) and DIPEA (40 µL, 233 µmol). The reaction mixture was stirred overnight at RT. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with saturated $NaHCO_3$ aq. (50 mL x2) and brine (50 mL) followed by drying over anhydrous Na_2SO_4. The solvent was removed in vacuon to yield **3-1** (73 mg, 80%) as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD): δ 8.46 (s, 1H), 8.14 (d, *J* = 8.0, 1H), 7.32 (d, *J* = 8.0, 1H), 6.83 (d, *J* = 8.8, 2H), 6.69 (s, 2H), 6.61 (d, *J* = 8.8, 2H), 4.26~4.34 (m, 1H), 3.96~4.01 (m, 1H), 3.60 (d, *J* = 11.6 Hz, 8H), 3.13~3.20 (m, 8H), 2.27 (t, *J* = 6.8 Hz, 2H), 2.02~2.15 (m, 1H), 1.91~1.99 (m, 1H) 1.62~1.69 (m, 12H), 1.50~1.62 (m, 8H), 1.44~1.47 (m, 67H). ^{13}C-NMR (600 MHz, CD_3OD): δ 175.26, 174.85, 173.80, 173.78, 173.33, 172.40, 170.72, 168.36, 158.23, 154.37, 137.96, 135.17, 130.24, 125.96, 125.15, 120.64, 120.63, 116.82, 114.21, 111.22, 103.70, 82.44, 82.42, 82.04, 82.03, 80.78, 66.76, 66.75, 56.55, 54.87, 54.86, 54.36, 40.81, 40.45, 40.44, 33.17, 32.57, 31.36, 31.34, 30.07, 30.44, 30.04, 29.09, 28.85, 28.55, 28.54, 28.47, 28.46, 24.54, 24.48, 24.27. HR-ESI-MS calcd for $C_{81}H_{120}N_7O_{23}$ $[M+H]^+$ = 1588.8430; obsd 1588.8435.

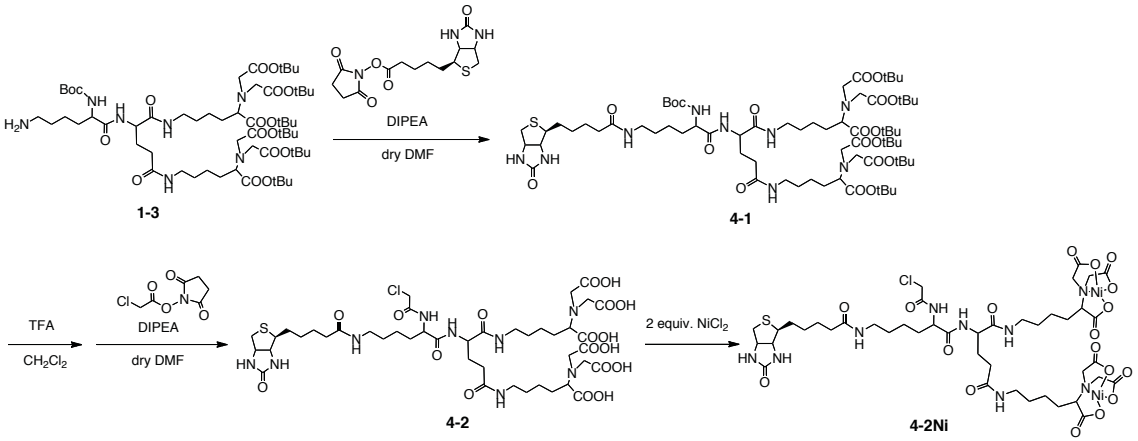
Compound 3

To a stirred solution of **3-1** (73 mg, 47 μmol) in dry CH_2Cl_2 (2 mL) was added dropwose TFA (1.5 mL) and the solution was stirred for 12h at RT. After removal of the solvent in vacuo, the residual TFA was azeotropically removed toluene (1.0 mL) to the crude deprotected product. To a solution of the crude product in dry DMF (2 mL) were added chloroacetic acid *N*-hydroxysuccinimide ester (9.1 mg, 47 μmol) and DIPEA (98 μL, 562 μmol) and the mixture was stirred for 3 h at RT. The reaction mixture was stirred for 2h at RT. After removal of the solvent in vacuo, the residue was purified by reverse-phase HPLC. The subsequent lyphilization afforded **3** (15 mg, 35% in 2steps from **3-1**) as a yellow solid. HPLC conditions: column; YMC triart-C18, 250 x 10 mm, mobile phase; CH_3CN (containing 0.1% TFA) / H_2O (containing 0.1% TFA) = 0 / 100 ~ 50 / 50 (linear gradient over 60 min), flow rate : 3 mL / min, detection UV wavelength; 220 nm. The purity of **3** was confirmed to be > 95% by HPLC analysis. ^{1}H-NMR (400 MHz, CD_3OD): δ 8.51 (s, 1H), 8.23 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz), 6.83 (s, 2H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.68 (d, *J* = 8.8 Hz, 2H), 4.23~4.36 (m, 2H), 4.13 (d, *J* = 7.2 Hz, 2H), 3.78 (d, *J* = 15.2 Hz, 8H), 3.62~3.70 (m, 2H), 3.42~3.70 (m, 2H), 3.13~3.21 (m, 4H), 2.28~2.33 (m, 2H), 2.03~2.16 (m, 1H), 1.72~1.94 (m, 9H), 1.38~1.59 (m, 10H), HR-ESI-MS calcd for $C_{54}H_{65}ClN_7O_{22}$ $[M+H]^+$ = 1198.3867; obsd 1198.3866

Compound 3-2Ni(II)

A purified **3** was dissolved in DMSO (100 μL). The concentration of **3** was determined to be 3.0 mM based on the UV absorbance at 494 nm using the extinction coefficient of fluorescein (ε_{494} = 75,000 M^{-1} · cm^{-1}).[S7] An aqueous $NiCl_2$ solution (6.0 mM, 100 μL) was added to give a stock solution of **3-2Ni(II)** (1.5 mM).

Synthesis of **4-2Ni(II)**



Scheme S4. Synthesis of **4-2Ni(II)**.

Compound 4-1

To a stirred solution of **1-3** (45 mg, 37 μmol) in dry DMF (3 mL) were added biotin succinimidyl ester (14 mg, 41 μmol) and DIPEA (7.8 μL, 45 μmol). The reaction mixture was stirred for 2h at RT. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with saturated $NaHCO_3$ aq. (50 mL x2) and brine (50 mL) followed by drying over anhydrous Na_2SO_4. The solvent was removed in vacuo to give **4-1** (48 mg, 90%) as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.48~4.54 (m, 1H), 4.31~4.42 (m, 2H), 4.12~4.20 (m, 1H), 3.40~3.74 (m, 10H), 3.16~3.38(m, 8H), 2.72~2.82(m, 1H), 2.05~2.32 (m, 6H), 1.51~1.75 (m, 20H), 1.42~1.46 (m, 67H). ^{13}C-NMR (600 MHz, CD_3OD): δ 173.31, 173.09, 172.64, 172.41, 172.29, 171.95, 170.77, 170.70, 163.97, 155.84, 81.18, 81.11, 80.80, 80.71, 79.68, 65.22, 64.98, 62.07, 60.09, 56.17, 54.53, 53.83, 53.64, 53.11, 40.63, 39.41, 39.38. 38.52, 35.17, 32.51, 32.25, 30.28, 29.95, 29.07, 28.54, 28.38, 28.22, 28.14, 28.13, 28.08, 28.03, 27.81, 27.63, 25.32, 23.31, 23.03, 22.32. HR-ESI-MS calcd for $C_{70}H_{124}N_9O_{19}S$ $[M+H]^+$ = 1426.8729; obsd 1426.8696.

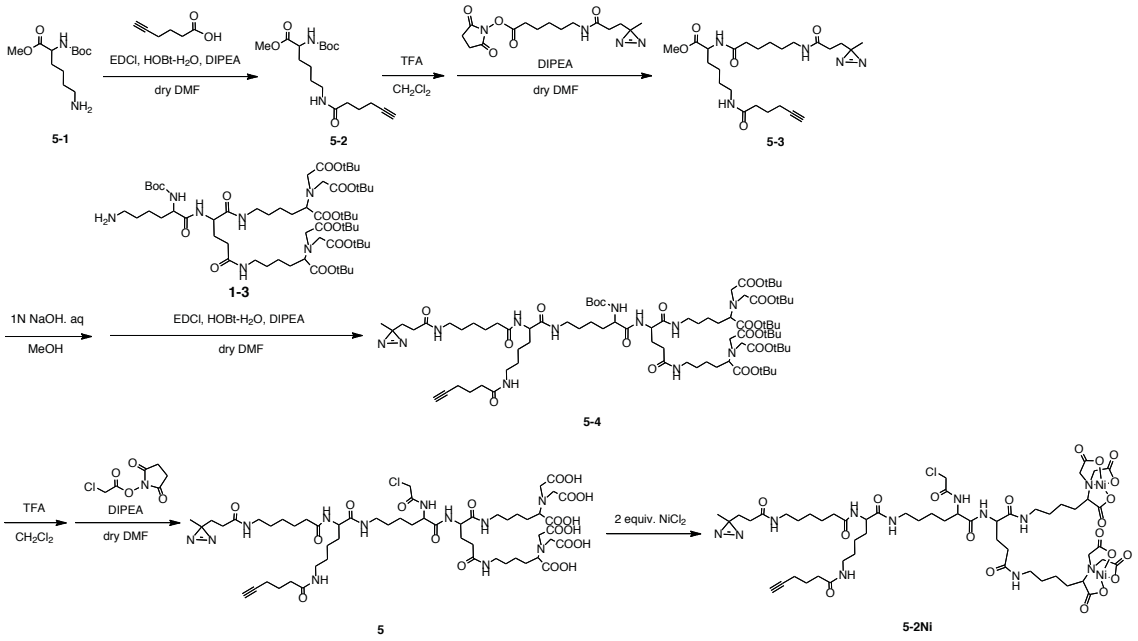
Compound 4

To a stirred solution of **4-1** (48 mg, 34 μmol) in dry CH_2Cl_2 (2 mL) was added dropwise TFA (1.5 mL) and the solution was stirred for 12 h at RT. After removal of the solvent in vacuo, the residual TFA was azeotropically removed with toluene (1.0 mL) to give the crude deprotected product. To a solution of the crude product in dry DMF (2 mL) were added chloroacetic acid *N*-hydroxysuccinimide ester (6.9 mg , 36 μmol) and DIPEA (6.8 μL, 39 μmol) and the mixture was stirred for 2 h at RT. The reaction mixture was stirred for 2h at RT under N_2 atmosphere. After removal of the solvent in vacuo, the residue was purified by reverse-phase HPLC. The subsequent lyphilization afforded **4** (7.3 mg, 17% in 2 steps from **4-1**) as colorless solid. HPLC conditions: column; YMC triart-C18, 250 x 10 mm, mobile phase; CH_3CN (containing 0.1% TFA) / H_2O (containing 0.1% TFA) = 0 / 100 ~ 40 / 60 (linear gradient over 40 min), flow rate : 3 mL / min, detection UV wavelength; 220 nm. The purity of **4** was confirmed to be > 95% by HPLC analysis. ^{1}H-NMR (400 MHz, CD_3OD): δ 4.87~4.52 (m, 1H), 4.33~4.25 (m, 3H), 4.14 (d, J = 5.2 Hz, 2H), 3.71 (d, J = 30 Hz, 8H), 3.56 (t, J = 6.8 Hz, 2H), 3.16 ~ 3.25 (m, 7H), 2.91~2.96 (m, 1H), 2.69~2.96 (m, 1H), 2.32 (t, J = 7.6 Hz, 2H), 2.22 (t, J = 7.6 Hz, 2H), 2.06~2.12 (m, 1H), 1.91~1.97 (m, 1H), 1.63~1.83 (m, 8H), 1.42~1.61 (m, 16H). ^{13}C-HMR (600 MHz, $CDCl_3$): δ 173.16, HR-ESI-MS calcd for $C_{43}H_{69}ClN_9O_{18}S$ $[M+H]^+$ = 1066.4164; obsd 1066.4135.

Compound 4-2Ni(II)

A purified **4** (4.3 mg, 4.0 μmol) was dissolved in DMSO (1 mL) . This solution was mixed with an aqueous $NiCl_2$ solution (8.0 mM, 1 mL) to give a stock solution of **4-2Ni(II)** (2.0 mM).

Synthesis of **5-2Ni(II)**



Scheme S5. Synthesis of **5-2Ni(II)**.

Compound 5-2

To a stirred solution of **5-1** (315 mg, 0.97 mmol) in dry DMF (5 mL) were added 5-hexynoic acid (130 mg, 1.16 mmol), EDCl (227 mg, 1.45 mmol), HOBt-H_2O (222 mg, 1.45 mmol) and DIPEA (840μL, 4.85 mmol). The reaction mixture was stirred overnight at RT. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with saturated $NaHCO_3$ aq. (50 mL x2) and brine (50 mL) followed by drying over anhydrous Na_2SO_4. The solvent was removed in vacuo to give **5-2** (361 mg, quant) as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.74 (s, 1H), 3.23~328 (m, 2H), 2.30 (t, J = 7.2 Hz, 2H), 2.24~2.28 (m, 2H), 1.97 (s, 1H), 1.77~1.90 (m, 2H), 1.62~1.70 (m, 1H), 1.50~1.55 (m, 2H), 1.46 (s, 9H), 1.34~1.43 (m, 2H). ^{13}C-HMR (600 MHz, $CDCl_3$): δ 173.16, 172.29, 155.45, 83.47, 79.81, 69.11,
53.08, 52.23, 38.93, 34.95, 32.28, 28.93, 28.24, 24.07, 22.48, 17.74. HR-ESI-MS calcd for $C_{18}H_{31}N_2O_5$ $[M+H]^+$ = 355.2227; obsd 355.2220.

Compound 5-3

To a stirred solution of **5-2** (35 mg, 97 μmol) in dry CH_2Cl_2 (2 mL) was added dropwose TFA (1.5 mL) and the solution was stirred for 1 h at 0°C. After removal of the solvent in vacuo, the residual TFA was azeotropically removed with toluene (1.0 mL) to give a crude deprotected product. To a solution of the crude product in dry DMF (2 mL) were added succinimidyl 6-(4,4'-azipentanamido)hexanoate (27 mg, 80 μmol) and DIPEA (5.4 μL, 31 μmol). The reaction mixture was stirred for 3 h at RT. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with 5% citric acid (50 mL x2) and brine (50 mL) followed by drying over Na_2SO_4. The

solvent was removed in vacuo to give **5-3** (30 mg, 64%) as colorless solid. ^{1}H-NMR(400 MHz, $CDCl_3$): δ 4.55~4.59 (m, 1H), 3.75 (s, 1H), 3.21~3.27 (m, 4H), 2.32 (t, *J* = 7.6, 2H), 2.22~2.28 (m, 4H), 1.96~2.00 (m, 3H), 1.81~1.88 (m, 3H), 1.74~1.77 (m, 4H), 1.64~1.69 (m, 3H), 1.55 (t, *J* = 7.2 Hz, 2H), 1.03 (s, 1H). ^{13}C-NMR(600 MHz, $CDCl_3$): δ 173.08, 173.06, 172.65, 171.42, 83.51, 69.26, 52.44, 51.82, 39.22, 38.72, 35.99, 35.11, 30.65, 30.09, 29.07, 28.96, 26.08, 25.63, 25.54, 24.92, 24.23, 22.34, 19.95, 17.87. HR-ESI-MS calcd for $C_{24}H_{40}N_5O_5$ $[M+H]^+$ = 478.3024; obsd 478.3024.

Compound 5-4

A solution of **5-3** (28 mg, 59 μmol) and 1N NaOH aq. (293 μL, 293 μmol) in MeOH (3 mL) was stirred overnight at 0°C. The solvent was removed in vacuo to give the crude deprotected product. To a solution of the crude product in dry DMF (3 mL) were added **1-3** (47 mg, 40 μmol), EDCl (12 mg, 60 μmol), HOBt-H_2O (9.2 mg, 60 μmol) and DIPEA (42 μL, 240 μmol). The reaction mixture was stirred overnight. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with 5% citric acid (50 mL x2) and brine (50 mL) followed by drying over anhydrous Na_2SO_4. The solvent was removed in vacuo to give **5-4** (55 mg, 85%) as colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.32~4.40 (m, 1H), 4.15~4.22 (m, 1H), 4.23~4.09 (m, 1H), 3.31~3.56 (m, 8H), 3.08~3.30 (m, 12H), 2.25~2.88 (m, 8H), 1.95~2.06 (m, 5H), 1.81~1.90 (m, 4H), 1.73~1.78 (m, 4H), 1.66~1.76 (m, 8H), 1.52~1.64 (m, 10H), 1.38~1.48 (m, 71H), 1.02 (s, 3H). ^{13}C-NMR(600 MHz, $CDCl_3$) : δ 173.54, 173.00, 172.67, 172.38, 172.32, 171.58, 171.55, 171.50,
170.82, 170.79, 170.74, 170.72, 81.23, 81.16, 80.83, 80.76, 69.25, 69.23, 65.24, 64.99, 54.62, 53.97, 53.90, 53.78, 52.99, 39.47, 39.29, 39.23, 38.91, 38.84, 35.98, 35.09, 32.26, 32.01, 31.93, 31.69, 30.58, 30.36, 30.24, 30.16, 30.15, 29.70, 29.07, 28.99, 28.55, 28.39, 28.24, 28.15, 28.13, 26.21, 25.59, 25.19, 24.35, 23.34, 23.04, 22.65, 21.98, 19.92, 17.95. HR-ESI-MS calcd for $C_{83}H_{145}N_{12}O_{21}$ $[M+H]^+$ = 1646.0642; obsd 1646.0641.

Compound 5

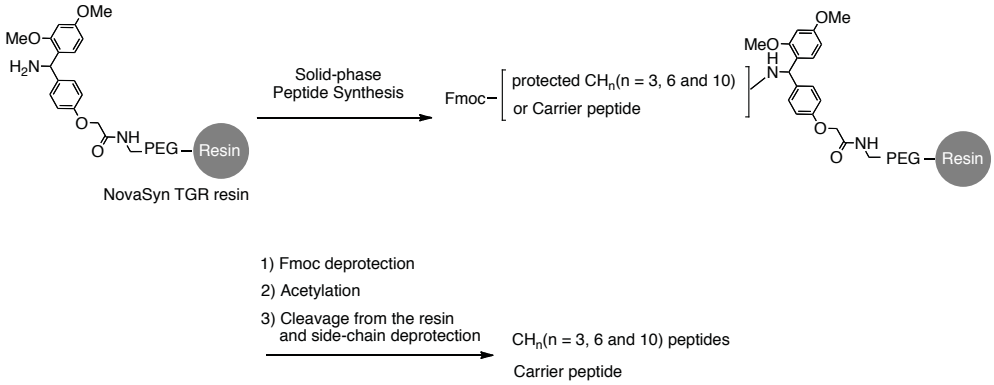
To a stirred solution of **5-4** (45 mg, 27 μmol) in dry CH_2Cl_2 (2 mL) was added dropwise TFA (1.5 mL) and the solution was stirred for 3 h at RT. After removal of the solvent in vacuo, the residual TFA was azeotropically removed with toluene (1.0 mL) to give the crude deprotected product. To a solution of the crude product in dry DMF (2 mL) were added chloroacetic acid *N*-hydroxysuccinimide ester (6.9 mg, 36 μmol) and DIPEA (6.9 μL, 39 μmol) and the mixture was stirred for 2 h at RT. After removal of the solvent in vacuo, the residue was purified by reverse-phase HPLC. The subsequent lyophilization afforded **5** (2.5 mg, 1.6 μmol, 5.9% in 2 steps from **5-4**) as colorless solid. HPLC conditions: column; YMC triart-C18, 250 x 10 mm, mobile phase; CH_3CN (containing 0.1% TFA) / H_2O (containing 0.1% TFA) = 0 / 100 ~ 50 / 50 (linear gradient over 50 min), flow rate : 3 mL / min, detection UV wavelength; 220 nm. The purity of **5** was confirmed to be > 95% by HPLC analysis. ^{1}H-NMR (400 MHz, CD_3OD): δ 4.23~4.30 (m, 3H), 4.15 (s, 2H), 3.63 (d, *J* = 27Hz, 8H), 3.47~3.50 (m, 2H), 3.12~3.24 (m, 10H), 2.19~2.32 (m, 9H), 2.07 (t, *J* = 8.0, 2H), 1.90~1.94 (m, 2H), 1.36~1.81 (m, 34H), 1.00 (s, 3H), HR-ESI-MS calcd for $C_{56}H_{90}ClN_{12}O_{20}$ $[M+H]^+$ = 1285.6083; obsd 1285.6077

Compound 5-2Ni(II)

A purified **5** (2.5 mg, 1.6 μmol) was dissolved in DMSO (1.8 mL). This solution was mixed with an aqueous $NiCl_2$ solution (4.0 mM, 1.8mL) to give a stock solution of **5-2Ni(II)** (1.0 mM).

Peptide Synthesis

The peptides were synthesized manually on Novasyn TGR resin (Novabiochem) by standard Fmoc-based solid-phase peptide synthesis protocol. Fmoc-Arg(Pbf)-OH, Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Trp(Boc)-OH were used as building blocks. Fmoc deprotection was performed with 20% piperidine in *N*-methylpyrrolidone (NMP), and 4-methyltrityl (Mtt) deprotection was performed in DCM containing 1% TFA and 5% triisopropylsilane (TIS). Coupling reactions were carried out with a mixture of Fmoc-amino acid (3 equiv), HOBt-H_2O (3 equiv), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 3 equiv), and DIPEA (6 equiv) in NMP. The coupling reaction of 4-Dimethylaminoazobenzene-4'-sulfonyl chloride(Dabsyl chloride) was carried out with a mixture of dabsyl chloride (3 equiv) and DIPEA (6 equiv) in CH_2Cl_2. All coupling and Fmoc and Mtt deprotection steps were monitored by the Kaiser test[S8]. Deprotection and cleavage from the resin was performed with 95% TFA containing 2.5% TIS, 2.5% H_2O. In the case of the Cys-containing peptide, TFA containing 1% triisopropylsilane (TIS), 2.5% ethanedithiol and 2.5% H_2O was used. The crude peptide was precipitated in *tert*-butyl methyl ether and purified by reverse-phase HPLC. HPLC conditions: colum; YMC-pack Triart-C18, 250 x 5 mm), CH_3CN (containing 0.1% TFA) / H_2O (containing 0.1% TFA) = 0 / 100 → 40 / 60 (linear gradient over 40 min), flow rate; 3 mL / min, UV detection wavelength ; 220 nm. Molecular weight of the peptide was confirmed by MALDI-TOF mass spectroscopy (positive mode). The purity of the peptides was confirmed to be > 95% by HPLC analysis.



Scheme S6. Synthesis of CHn(n = 3, 6 and 10) peptides and Carrier peptide.

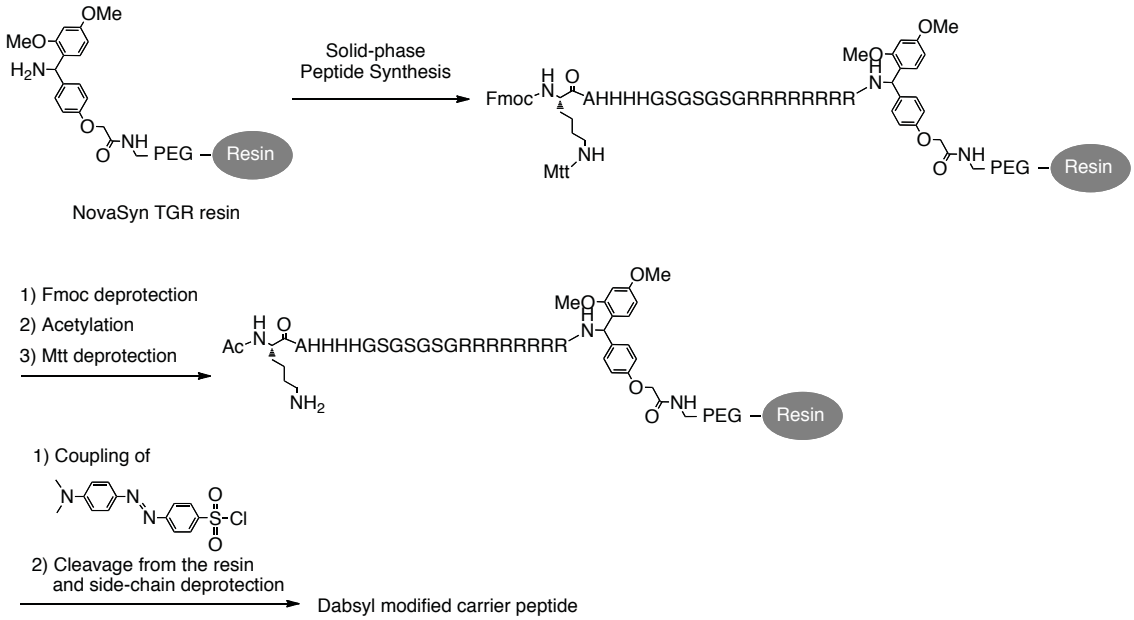
CH3 peptide: Ac-WACHHH-NH_2 calcd for $C_{37}H_{47}N_{14}O_7S$ = 831.34 $[M+H]^+$; obsd 830.01

CH6 peptide: Ac-WACHHHHHH-NH_2 calcd for $C_{55}H_{68}N_{23}O_{10}S$ = 1242.52 $[M+H]^+$; obsd 1242.10

CH10 peptide: Ac-WACHHHHHHHHHH-NH_2 calcd for $C_{79}H_{96}N_{35}O_{14}S$ = 1790.76$[M+H]^+$; obsd 1790.28

Carrier peptide: Ac-WAHHHHGSGSGSGRRRRRRRR-NH_2 calcd for $C_{105}H_{172}N_{55}O_{25}S$ = 2603.39 $[M+H]^+$; obsd

2603.74



Scheme S7. Synthesis of dabsyl-appended carrier peptide.

Dabsyl-appended carrier peptide: Ac-K(dabsyl)AHHHHGSGSGSGRRRRRRRR-NH_2 calcd for $C_{114}H_{186}N_{57}O_{28}S$ = 2833.46 $[M+H]^+$; obsd 2833.04

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