Supplementary Information

Facile preparation of functional cycloalkynes by an azide-to-cycloalkyne switching approach

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Contents

General Remarks

All reactions were performed in dry glasswares under atmosphere of argon, unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm) silica-gel plates (Merck Chemicals, Silica Gel 60 F_{254}, Cat. No. 1.05715). Column chromatography was conducted using silica-gel (Kanto Chemical Co., Inc., Silica Gel 60N, spherical neutral, particle size 40–50 μm, Cat. No. 37563-85) or using Biotage® ZIP sphere cartridge [silica] or Biotage® SNAP Ultra cartridge [silica] with medium pressure liquid chromatography (Yamazen, W-Prep 2XY A-type). Preparative TLC was performed on silica-gel (Wako Pure Chemical Industries, Ltd., Wakogel® B-5F, Cat. No. 230-00043, or Merck Chemicals, Silica Gel 60 PF_{254} for preparative TLC, Cat. No. 1.07747). Melting points (Mp) were measured on a YANACO MP-J3 instrument or on an OptiMelt MPA100 (Stanford Research Systems) and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AVANCE 500 spectrometer at 500 or 126 MHz, respectively. ^{19}F NMR spectra were obtained with a Bruker AVANCE 400 spectrometer at 376 MHz. All NMR measurements were carried out at 23 ºC. $CDCl_3$ or CD_3OD was used as a solvent for obtaining NMR spectra. Chemical shifts (δ) are given in parts per million (ppm) downfield from $(CH_3)_4Si$ (δ 0.00 for ^{1}H NMR and ^{13}C NMR in $CDCl_3$) or the solvent peak (δ 3.31 for

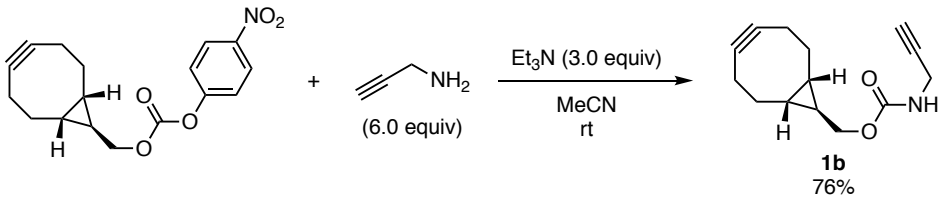
^{1}H NMR in CD_3OD and δ 49.2 for ^{13}C NMR in CD_3OD) as an internal reference, or α,α,α-trifluorotoluene (δ –63.0 ppm for ^{19}F NMR in $CDCl_3$) as an external standard with coupling constants (*J*) in hertz (Hz). The abbreviations s, d, t, q, m, and br signify singlet, doublet, triplet, quartet, multiplet, and broad, respectively. IR spectra were measured by diffuse reflectance method on a Shimadzu IRPrestige-21 spectrometer attached with DRS-8000A with the absorption band given in cm^{-1}. High-resolution mass spectra (HRMS) were measured on a Bruker micrOTOF mass spectrometer under positive electrospray ionization (ESI^+) conditions.

N-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)biotinamide (**2c**) (Cat. No. A2523) and 2-(2-(2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethyl azide (**2d**) (Cat. No. A2727) were purchased from Tokyo Chemical Industry Co., Ltd. Alexa Fluor 555 azide triethylammonium salt (**2i**) (Cat. No. A20012) and Alexa Fluor 488 azide (**2k**) (A10266) were purchased from ThermoFisher Scientific, Inc. Polymer-bound triphenylphosphine (PS–TPP) (Cat. No. 93093) was purchased from Sigma–Aldrich Japan. All chemical reagents used were commercial grade and used as received, unless otherwise noted. (1α,8α,9α)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate,[S1] 2-(cyclooctyn-3-yloxy)ethyl (4-nitrophenyl) carbonate,[S2] 3-(4-tosyl-4,8-diazacyclononyn-8-ylcarbonyl)propionic acid,[S3] 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl *N*-(2-propyn-1-yl)carbamate (**1a**),[S4] 4-(methoxycarbonyl)benzyl azide (**2a**),[S5] 4-(2-(2-(6-chlorohexyloxy)ethoxy)-ethylaminocarbonyl)benzyl azide (**2b**),[S6] (11-oxo-2,3,6,7-tetrahydro-1*H*,5*H*,11*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-9-yl)methyl azide (**2e**),[S7] 3-(2,6-bis(ethoxycarbonyl)-4,4-difluoro-1,3,5,7-tetramethyl-3a,4a-diaza-4-bora-*s*-indacen-8-yl)-4-methoxyphenyl azide (**2f**),[S8] 3-(4-(3,6-bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido)propyl azide (**2g**),[S9] 2-(2-(2-(2-(4-(3,6-bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido)ethoxy)ethoxy)ethoxy)ethyl azide (**2h**),[S6] 1-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-3-(4-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-3-carboxyphenyl)thiourea (**2j**)[S10] and *tert*-butyl (2-(2-(6-chlorohexyloxy)ethoxy)ethyl)carbamate (**S1**)[S11] were prepared according to the reported method.

CAUTION! **Azido-containing compounds are presumed to be potentially explosive. Although we have never experienced such an explosion with azido compounds used in this study, all manipulations should be carefully carried out behind a safety shield in a hood.**

Chemical Experiments

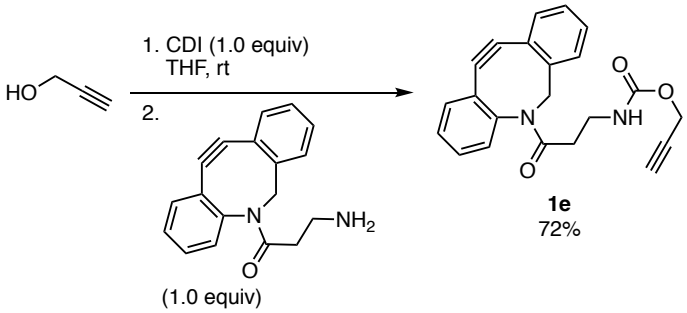
A typical procedure for preparation of diynes using 4-nitrophenyl carbonates



To a solution of (1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate[S1] (811 mg, 2.57 mmol) and propargylamine (849 mg, 15.4 mmol) dissolved in MeCN (26 mL) was added triethylamine (780 mg, 7.71 mmol) at room temperature. After stirring for 150 min at the same temperature, the mixture was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (60 mL), washed three times with an aqueous solution of NaOH (1 M, 60 mL and 30 mL × 2), washed three times with brine (60 mL × 2 and 30 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica-gel 30 g, *n*-hexane/EtOAc = 4/1) to give (1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-(2-propyn-1-yl)carbamate (**1b**) (454 mg, 1.96 mmol, 76.4%) as a colorless solid.

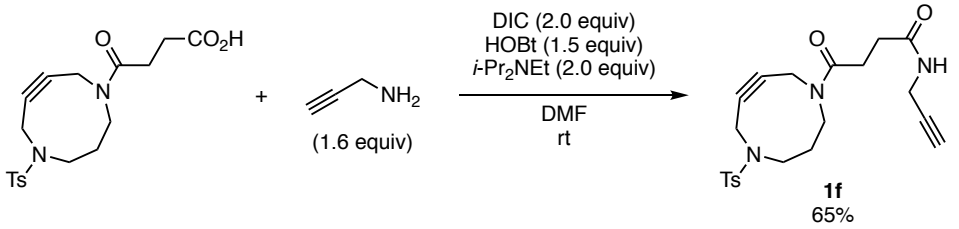
According to this procedure, (1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-(2-(2-(2-propyn-1-yloxy)ethoxy)ethyl)carbamate (**1c**) (295 mg, 92.4%) was prepared using 2-(2-(2-propyn-1-yloxy)ethoxy)ethylamine instead of propargylamine, and 2-(cyclooctyn-3-yloxy)ethyl *N*-(2-propyn-1-yl)carbamate (**1d**) (1.00 g, 94.8%) was prepared using 2-(cyclooctyn-3-yloxy)ethyl (4-nitrophenyl) carbonate[S2] instead of (1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate.

*Preparation of diyne **1e***



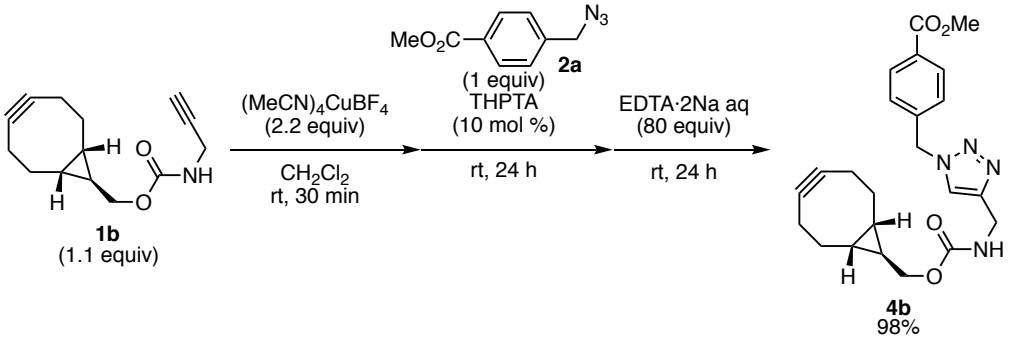
To a solution of 1,1'-carbonyldiimidazole (CDI) (32.4 mg, 0.200 mmol) dissolved in THF (2.0 mL) was added propargyl alcohol (11.2 mg, 0.200 mmol) at room temperature. After stirring for 3 h at the same temperature, to the mixture was added 3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropyl)amine (56.0 mg, 0.204 mmol) and stirred for 24 h at the same temperature. After an addition of H_2O (30 mL), the mixture was extracted with CH_2Cl_2 (20 mL × 3), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (*n*-hexane/EtOAc = 1/1) to give 2-propyn-1-yl *N*-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropyl)carbamate (**1e**) (51.9 mg, 0.145 mmol, 72.5%) as a colorless solid.

*Preparation of diyne **1f***



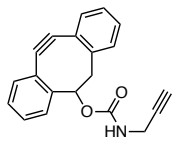
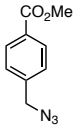
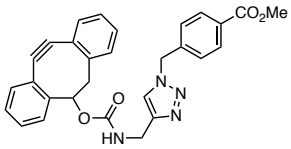
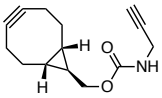
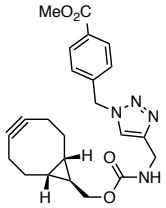
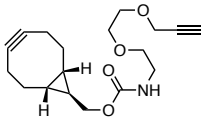
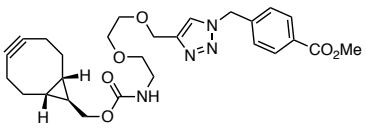
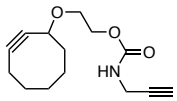
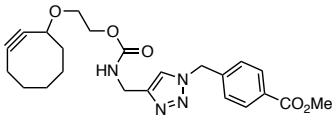
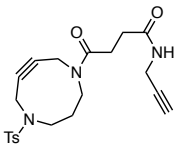
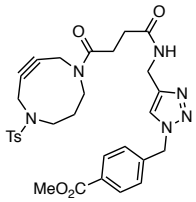
To a solution of 3-(4-tosyl-4,8-diazacyclononyn-8-ylcarbonyl)propionic acid[S3] (227 mg, 0.600 mmol), propargylamine (60 μL, 0.94 mmol), and 1-hydroxybenzotriazole (HOBt) (122 mg, 0.903 mmol) dissolved in DMF (12 mL) were successively added *N*,*N*'-diisopropylcarbodiimide (DIC) (0.19 mL, 1.2 mmol) and *N*,*N*-diisopropylethylamine (0.21 mL, 1.2 mmol) at room temperature. After stirring for 20 h at the same temperature, to the mixture was added an aqueous saturated solution of NH_4Cl (50 mL). The mixture was extracted three times with Et_2O (50 mL and 25 mL × 2), washed three times with brine (25 mL × 3), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica-gel 10 g, *n*-hexane/EtOAc = 25/75 to 10/90) to give *N*-(2-propyn-1-yl) 3-(4-tosyl-4,8-diazacyclononyn-8-ylcarbonyl)propionamide (**1f**) (163 mg, 0.392 mmol, 65.4%) as a colorless solid.

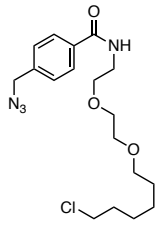
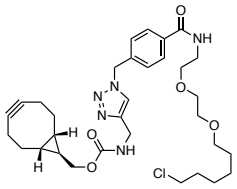
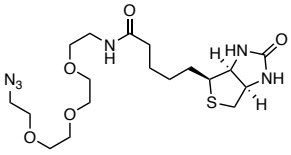
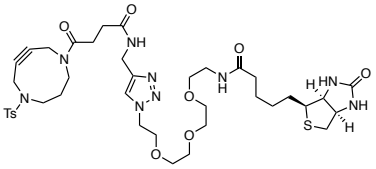
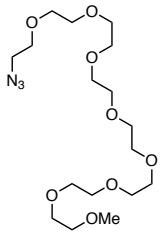
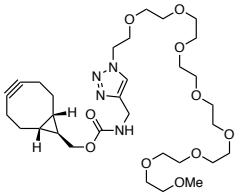
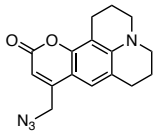
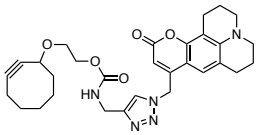
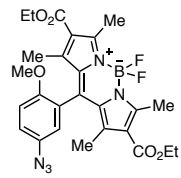
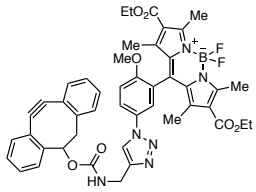
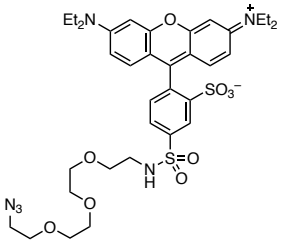
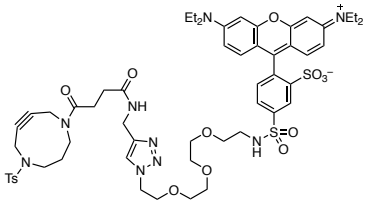
A typical procedure for selective click reaction of the terminal alkyne moiety of diynes



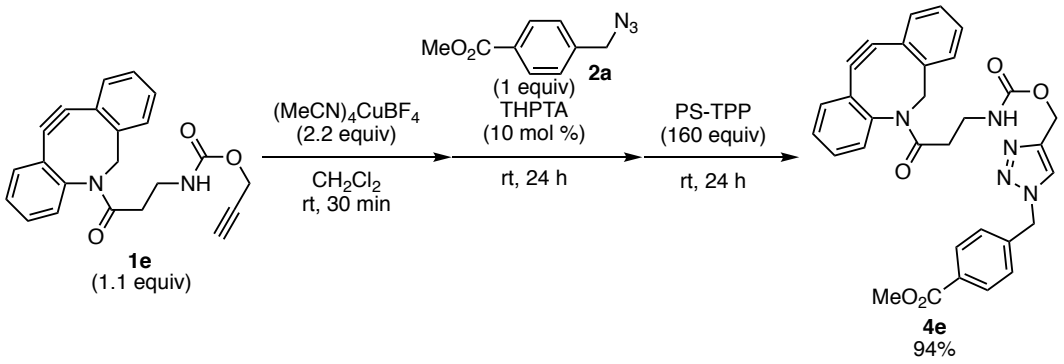
A mixture of (1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-(2-propyn-1-yl)carbamate (**1b**) (25.5 mg, 0.110 mmol) and tetrakis(acetonitrile)copper(I) tetrafluoroborate (69.5 mg, 0.221 mmol) was dissolved in CH_2Cl_2 (2.0 mL) at room temperature. After stirring for 30 min at the same temperature, to the reaction mixture were successively added a solution of 4-(methoxycarbonyl)benzyl azide (**2a**) (19.3 mg, 0.101 mmol) dissolved in CH_2Cl_2 (3.0 mL) and tris((1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)methyl)amine (THPTA) (4.4 mg, 10 μmol). After stirring for 24 h at the same temperature, to the mixture were added CH_2Cl_2 (10 mL) and an aqueous solution of ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid disodium salt (EDTA·2Na) (0.10 M, 80 mL, 8.0 mmol). After stirring for 24 h at the same temperature, the reaction mixture was extracted three times with CH_2Cl_2 (20 mL × 3), and the combined organic extract was dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (CH_2Cl_2/MeOH = 10/1) to give 4-((1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethoxycarbonylaminomethyl)-1-(4-(methoxycarbonyl)benzyl)-1*H*-1,2,3-triazole (**4b**) (42.0 mg, 99.4 μmol, 98.5%) as a colorless solid.

According to this procedure, cycloalkynes **4a**, **4c**, **4d**, **4f**–**k**, and **4m** were prepared using corresponding diynes **1** and azides **2**. The results are summarized in the following tables.

Entry	Diyne **1**	Azide **2**	Cycloalkyne **4**	Yield (%)
1	 **1a**	 **2a**	 **4a**	92
2	 **1b**	**2a**	 **4b**	98
3	 **1c**	**2a**	 **4c**	99
4	 **1d**	**2a**	 **4d**	94
5	 **1f**	**2a**	 **4f**	92

Entry	Diyne **1**	Azide **2**	Cycloalkyne **4**	Yield (%)
6	**1b**	 **2b**	 **4g**	89
7	**1f**	 **2c**	 **4h**	77
8	**1b**	 **2d**	 **4i**	80
9	**1d**	 **2e**	 **4j**	98
10	**1a**	 **2f**	 **4k**	69
11	**1f**	 **2h**	 **4m**	98

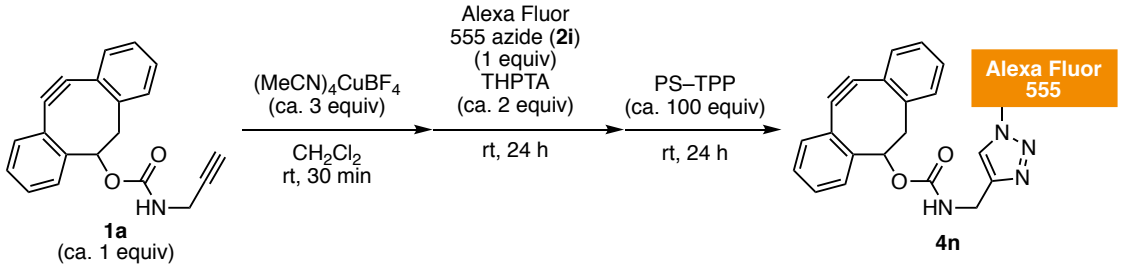
A typical procedure for selective click reaction of the terminal alkyne moiety of diynes using PS–TPP instead of EDTA



A mixture of 2-propyn-1-yl *N*-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropyl)carbamate (**1e**) (39.5 mg, 0.110 mmol) and tetrakis(acetonitrile)copper(I) tetrafluoroborate (69.4 mg, 0.221 mmol) was dissolved in CH_2Cl_2 (2.0 mL) at room temperature. After stirring for 30 min at the same temperature, to the reaction mixture were successively added a solution of 4-(methoxycarbonyl)benzyl azide (**2a**) (19.1 mg, 0.100 mmol) dissolved in CH_2Cl_2 (3.0 mL) and tris((1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)methyl)amine (THPTA) (4.4 mg, 10 μmol). After stirring for 24 h at the same temperature, to the mixture were added CH_2Cl_2 (20 mL) and polymer-bound triphenylphosphine (PS–TPP) (ca. 3 mmol/g, 5.3 g, ca. 16 mmol). After stirring for 24 h at the same temperature, the reaction mixture was filtered through a pad of Celite, and then the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (CH_2Cl_2/MeOH = 15/1) to give 4-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropylaminocarbonyloxymethyl)-1-(4-(methoxycarbonyl)benzyl)-1*H*-1,2,3-triazole (**4e**) (51.8 mg, 94.3 μmol, 94.3%) as a colorless solid.

According to this procedure, 1-(3-(4-(3,6-bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido)propyl)-4-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropylaminocarbonyloxymethyl)-1*H*-1,2,3-triazole (**4l**) (21.4 mg, 86.8%) was prepared using 3-(4-(3,6-bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido)propyl azide (**2g**) instead of **2a**.

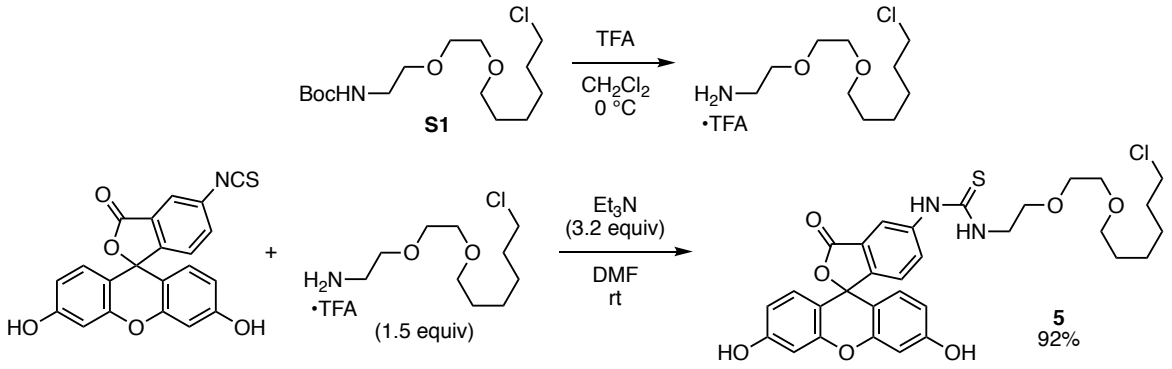
*Preparation of Alexa Fluor 555–DBCO **4n***



A mixture of 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl *N*-(2-propyn-1-yl)carbamate (**1a**) (16.6 mg, 55.1 μmol) and tetrakis(acetonitrile)copper(I) tetrafluoroborate (47.1 mg, 0.150 mmol) was dissolved in CH_2Cl_2 (2.0 mL) at room temperature. After stirring for 30 min at the same temperature, 10 μL of the reaction mixture containing ca. 0.28 μmol of copper-protected **1a** was added to a solution of Alexa Fluor 555 azide triethylammonium salt (**2i**) (0.25 mg, ca. 0.29 μmol, M_w ~850) and tris((1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)methyl)amine (THPTA) (0.23 mg, 0.53 μmol) dissolved in CH_2Cl_2 (90 μL). After stirring for 24 h at the same temperature, to the mixture were added CH_2Cl_2 (0.4

mL) and polymer-bound triphenylphosphine (PS–TPP) (ca. 3 mmol/g, 10 mg, ca. 30 μmol). After stirring for 24 h at the same temperature, the reaction mixture was filtered through a cotton plug, and then the residue was washed with CH_2Cl_2 (0.5 mL × 5). The resulting residue was extracted with MeOH (0.5 mL × 5), and the filtrate was concentrated under reduced pressure. The obtained pink solid was used as Alexa Fluor 555–DBCO **4n** for biological experiments without further purification.

*Preparation of fluorescein–HaloTag ligand **5***



To a solution of *tert*-butyl (2-(2-(6-chlorohexyloxy)ethoxy)ethyl)carbamate (**S1**)[S11] (55.0 mg, 0.170 mmol) dissolved in CH_2Cl_2 (3.5 mL) was added TFA (1.0 mL) at 0 °C. After stirring for 3 h at the same temperature, the mixture was concentrated under reduced pressure. To a solution of the crude mixture dissolved in DMF (2.0 mL) were added triethylamine (50 μL, 0.36 mmol) and 4-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-3-carboxyphenyl isothiocyanate (44.0 mg, 0.113 mmol) at room temperature. After stirring for 20 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (silica-gel 8 g, CH_2Cl_2/MeOH = 15/1) to give 1-(2-(2-(6-chlorohexyloxy)ethoxy)ethyl)-3-(4-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-3-carboxyphenyl)thiourea (**5**) (63.6 mg, 0.104 mmol, 91.8%) as a yellow solid.

Biological Experiments

Plasmid vectors

The vectors of GST-HaloTag for the recombinant protein expression in *E. coli* cells and HaloTag-TM for the expression of HaloTag with transmembrane domain in mammalian cells were constructed previously.[S6,S12]

Production of recombinant GST-HaloTag protein in E. coli[S12]

E. coli strain Rosetta (DE3) pLysS cells (Novagen, Merck Chemicals Ltd., Nottingham, UK) were transformed with the pGEX6P-1-HaloTag vector,[S6] and cultured in LB media containing 50 mg L^{-1} Carbenicillin (Nacalai Tesque, Kyoto, Japan) and 34 mg L^{-1} chloramphenicol (Nacalai Tesque). Expression was induced by the addition of isopropyl β-D-thiogalactopyranoside (final concentration at 1 mM) (Nacalai Tesque), when the culture had reached an OD_{600} of approximately 0.8. After induction for 16 h at 30 °C, the cells were collected by centrifugation at 4,500 g for 15 min, and frozen in liquid N_2. After thawing, the cells were suspended in cell lysis buffer containing 20 mM HEPES-KOH (pH 8.0), 200 mM NaCl, 2 mM tris(2-carboxyethyl)phosphine hydrochloride (Nacalai Tesque), 10% glycerol (Nacalai Tesque), and 1% Triton X-100, and then lysozyme (TCEP; Nacalai Tesque) was added to the cell lysate, which were incubated on ice for 30 min. $MgCl_2$ (final concentration at 10 mM) and DNase I (final concentration of approximately 20 μg mL^{-1}) were added into the cell lysate, and incubation was continued for 1 h at 4 °C. Cell debris and larger particles were removed by centrifugation at 8,000 g for 20 min at 4 °C, and the supernatant was then filtered through a 0.45-μm filter. The supernatant of the cell lysate containing the GST-HaloTag protein was applied onto a GST-Accept COSMOGEL (Nacalai Tesque), which had been pre-equilibrated with cell lysis buffer. After excessive washing of the resin with PBS (TAKARA BIO Inc.), the bound GST-HaloTag protein was subjected to the chemical modification. To analyze the concentration of the GST-HaloTag protein, we performed SDS-PAGE. The protein sample was diluted by 1:1 with 2× SDS sample loading buffer (Nacalai Tesque), heated for 5 min at 98 °C, and then loaded onto the gel. The proteins were stained with Coomassie brilliant blue (CBB) rapid stain kit (Nacalai Tesque). The concentration of the recombinant proteins was determined by comparing with bovine serum albumin (Fraction V; Nacalai Tesque) as the standard.

Chemical modification of GST-HaloTag

GST-HaloTag (total 1.0 nmol in 200 μL of reaction mixture), bound on the GST-Accept resin (bed volume; 15 μL), was incubated with 0.3% H_2O_2 and 100 μM of azido-HaloTag ligand **2b** in PBS overnight at 4 °C. The azide-incorporated GST-HaloTag protein bound on the resin (azido-GST-HaloTag-resin) was washed with PBS, and then reacted with 50 μM of copper-protected diyne **1c** (**1c-*Cu***) in the presence of 250 μM of $CuSO_4$, 500 μM of THPTA, and 2.5 mM of sodium ascorbate for 20 min at room temperature. **1c-*Cu*** was prepared by reaction of 200 mM of diyne **1c** and 400 mM of $(MeCN)_4CuBF_4$ in DMF for 30 min at room temperature. The reacted GST-HaloTag-resin was washed

with PBS containing 50 mM of EDTA for 6 h at room temperature, and then mixed with 50 mM of EDTA and 100 μM of fluorescein azide **2j** overnight at room temperature. As a positive control, GST-HaloTag-resin was reacted with 100 μM of fluorescein–HaloTag ligand **5**. The labeled GST-HaloTag-resins were washed with PBS, and then were excised from GST by the addition of PreScission protease (GE Healthcare UK Ltd, Buckinghamshire, UK) in 50 mM Tris-HCl containing 150 mM NaCl, 1 mM TCEP and 1 mM EDTA to the resin and incubation overnight at 4 °C. The eluates were subjected to SDS-PAGE and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analyses.

Chemical modification of antibody

Cetuximab, an antibody against human EGF receptor, was obtained from Merck (Darmstadt, Germany). Incorporation of azide on sugar chain of antibody was performed by SiteClick™ Antibody Labeling Kit (ThermoFisher Scientific, Inc., Waltham, Massachusetts, USA) according to the manufacture's protocol. The azido-incorporated antibody (approximately 20 μg) was diluted in 50 μL of PBS, and mixed with Pierce™ Protein A Plus Agarose (bed volume; 10 μL) (ThermoFisher Scientific, Inc.) for 1 h at room temperature. The azido-antibody bound on Protein A Plus Agarose was reacted with 200 μM of **1c-*Cu*** in the presence of 250 μM of $CuSO_4$, 500 μM of THPTA, and 2.5 mM of sodium ascorbate for 20 min at room temperature. The antibody on the agarose was washed with PBS containing 50 mM of EDTA for 6 h at room temperature, and then mixed with 50 mM of EDTA and 200 μM of Alexa Fluor 488 azide (**2k**) overnight at room temperature. After a wash with PBS, the labeled antibody was eluted with 100 mM glycine buffer (pH 3.0) containing 0.1% EMPIGEN BB (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), and immediately neutralized with 1 M Tris-HCl (pH 8.0). The eluate was incubated for 5 min at 98 °C with 1× SDS sample loading buffer, and subjected to SDS-PAGE analysis.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis was carried out under reducing conditions using a 5–20% polyacrylamide gel (ATTO, Tokyo, Japan). The gels were directly visualized by laser-scanning in a fluorescence imaging analyzer Typhoon 9410 (GE Healthcare). The gels were also stained with CBB rapid stain kit (Nacalai Tesque) or One-step Ruby (APRO SCIENCE, Tokushima, Japan).

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS)

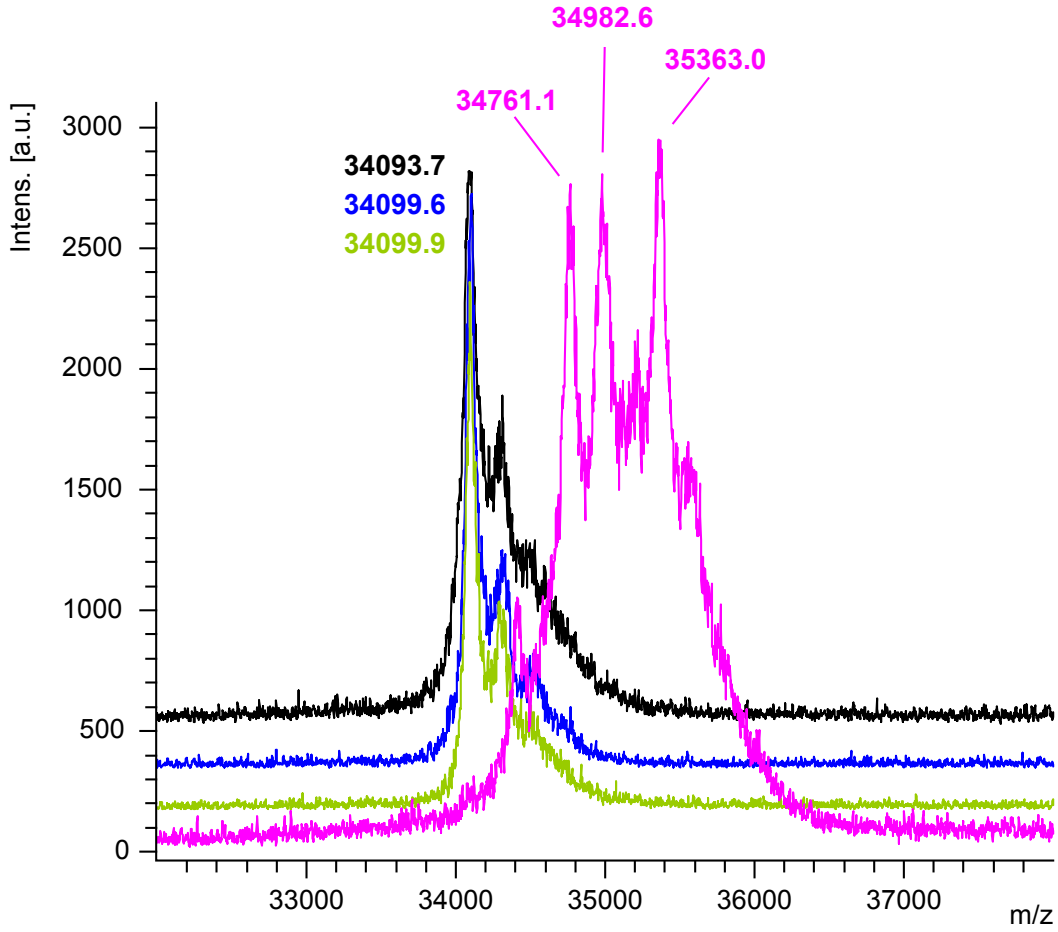
MALDI-TOF-MS was performed on an ultrafleXtreme TOF/TOF mass spectrometer (Bruker Daltonics Inc., Massachusetts, USA). The accelerating voltage in the ion source was set to 20 kV. Data were acquired in the positive linear mode of operation. Time-to-mass conversion was achieved by external calibration using standards of trypsinogen (*m*/*z* 23982) and protein A (*m*/*z* 44613, 22307) with/without cetuximab (*m*/*z* 151800). The matrix for proteins was sinapic acid (SA, M_w = 224; Bruker Daltonics Inc.). Saturated SA matrix solution was prepared in a 30% (v/v) solution of acetonitrile in

water containing 0.1% trifluoroacetic acid. The matrix (2 μL) was mixed with a solution (2 μL) of the labeled HaloTag protein (approximately 3.5 μg μL^{-1}) or the labeled antibody (approximately 3.0 μg μL^{-1}), and 0.5 μL of the mixture was applied on a steel sample plate (MTP AnchorChip 384 BC target plate; Bruker Daltonics Inc.). The mixture was allowed to air dry before being introduced into the mass spectrometer.

Fluorescence Labeling of the Living Cells

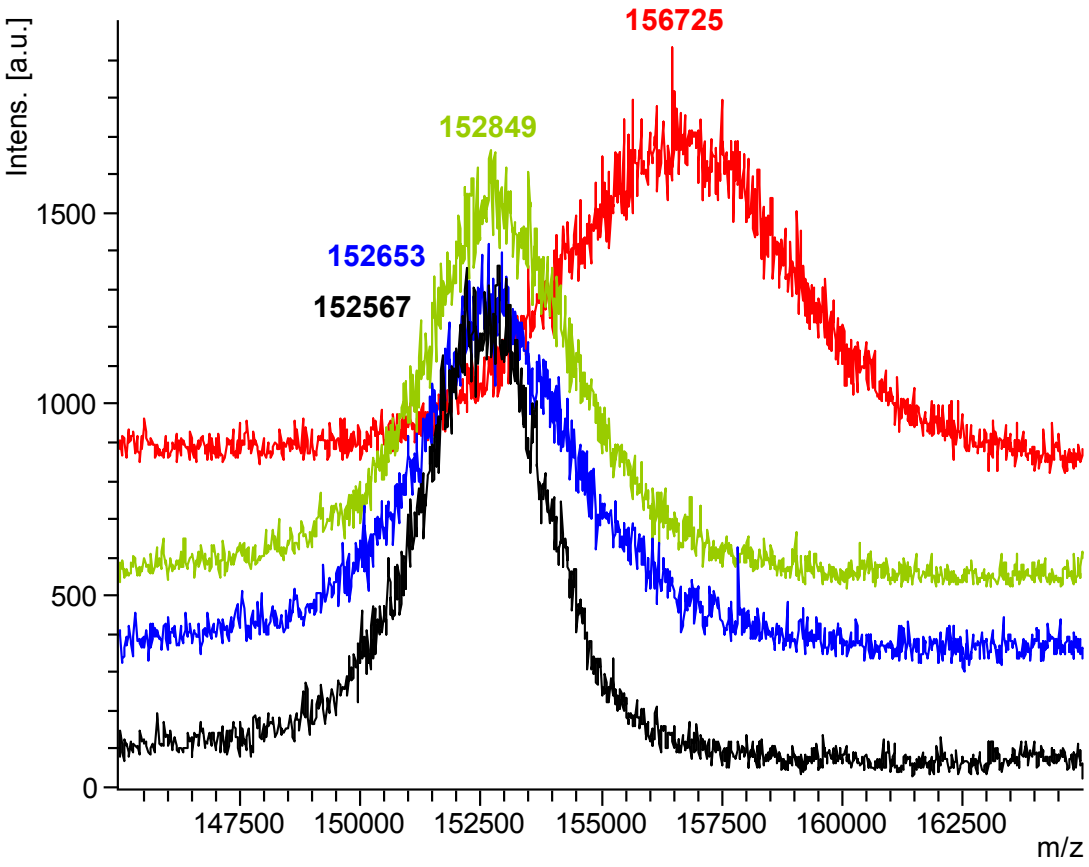
HEK293 cells were routinely maintained in a 5% CO_2, water-saturated atmosphere, and grown in low-glucose Dulbecco's Modified Eagle Medium (DMEM; Nacalai Tesque) supplemented with 10% Fetal Bovine Serum (FBS; Gibco, ThermoFisher Scientific, Inc.), 100 U mL^{-1} of penicillin (Nacalai Tesque), and 100 μg mL^{-1} of streptomycin (Nacalai Tesque). The cells were cultured on 8-well chamber slides (Matsunami, Osaka, Japan), and transfected with the pCAGIPuro vector harboring HaloTag-TM using polyethylenimine MAX (Polysciences Inc., *Pennsylvania,* USA). The cells were washed with pre-warmed DMEM once, and then incubated with DMEM supplemented with 10 μM of azido-HaloTag ligand **2b** for 30 min at 37 °C. After a wash with pre-warmed DMEM, the cells were incubated with 1 μM of Alexa Fluor 555–DBCO **4n** or Alexa Fluor 555 azide triethylammonium salt (**2i**) for 30 min at 37 °C. After incubation with pre-warmed fresh DMEM10%FBS for 30 min, the cells were fixed with 4% paraformaldehyde in phosphate buffer (Nacalai Tesque) for 10 min at room temperature. The fixed cells were incubated with PBS containing 0.1% Triton X-100 and Hoechst 33342 (1 μg mL^{-1}) for 1 h at room temperature to stain nuclei. After a wash with PBS followed by a wash with ultrapure water, the slides were mounted with ProLong Diamond (Thermo fisher Scientific, Massachusetts, USA). Fluorescence images were obtained with a confocal laser microscopy LSM800 (Carl Zeiss Microscopy, Oberkochen, Germany) equipped with 63× Plan-Apochromat NA 1.40 objective lens and imaging software (ZEN 2.3 system). Images were imported into Photoshop (Ver. CC 2018; Adobe) for cropping and linear contrast adjustment.

Supplemental Figures

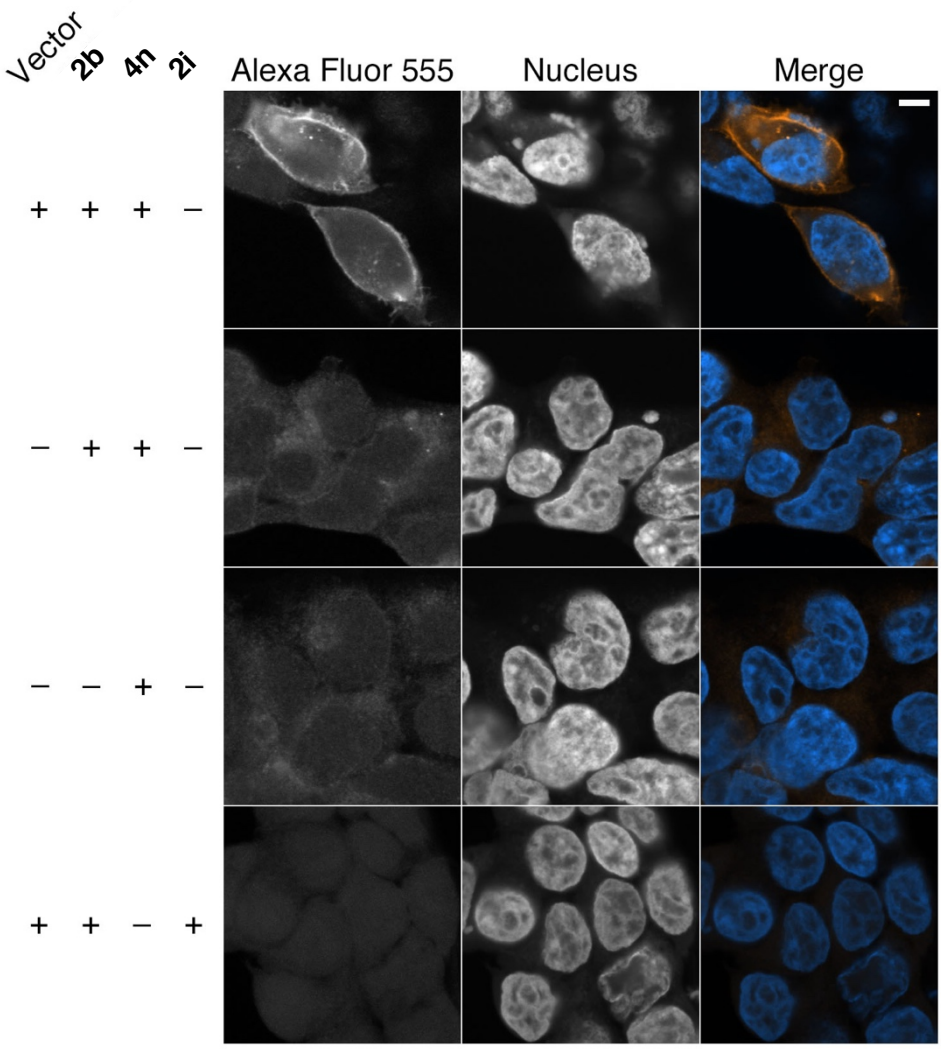


Mass spectra of the HaloTag proteins modified with azido-HaloTag ligand **2b**, **1c-*Cu***, and fluorescein azide **2j**.

The each modified GST-HaloTag was digested with the PreScission protease overnight at 4 °C to elute the modified HaloTag from the resin. The eluted proteins were analyzed by MALDI-TOF-MS. Mass spectrum of the unlabeled HaloTag is shown as a black line; the HaloTag protein reacted with fluorescein azide **2j**, blue line; the HaloTag protein reacted with **1c-*Cu*** and fluorescein azide **2j**, green line; the HaloTag protein reacted with azido-HaloTag ligand **2b** followed by with Cu-protected **1c-*Cu*** and fluorescein azide **2j**, pink line. The mass of the unlabeled HaloTag was observed at *m/z* 34094 as a major peak matched with its calculated value of 34336 (black line). The mass of the HaloTag treated with fluorescein azide **2j** and/or **1c-*Cu*** (blue and green lines) was almost the same as that of the unlabeled HaloTag (black line), indicating no non-specific modification. After incorporation of azido-HaloTag ligand **2b** (M_w = 438) into the HaloTag protein, the azide-incorporated HaloTag was reacted with **1c-*Cu*** followed with fluorescein azide **2j** (M_w = 608), resulting in that the mass of the fluorescently labeled HaloTag was observed at *m/z* 35363 as a major peak matched with its calculated value of 35569 (pink line). Thus, the increase of mass was in good agreement with the calculated mass value of the modified HaloTag protein.



Mass spectra of azido-incorporated antibodies modified with **1c-*Cu*** and Alexa Fluor 488 azide (**2k**). Mass spectrum of intact antibody is shown as a black line; antibody mixed with Alexa Fluor 488 azide (**2k**), blue line; antibody mixed with **1c-*Cu*** and Alexa Fluor 488 azide (**2k**), green line; antibody possessing azides on its sugar chain mixed with **1c-*Cu*** followed with Alexa Fluor 488 azide (**2k**), red line. The mass of the antibodies mixed with Alexa Fluor 488 azide (**2k**) and/or **1c-*Cu*** (blue and green lines) was almost the same as that of the intact antibody (black line), indicating no non-specific modification. After incorporation of azides on the sugar chains of antibody, the azide-incorporated antibody was reacted with **1c-*Cu*** followed with Alexa Fluor 488 azide (**2k**), resulting in that the mass of the fluorescently labeled antibody was observed at the larger *m/z* value as a major peak (red line), indicating specific modification.

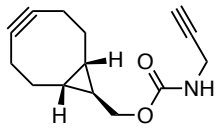


Fluorescent labeling of HaloTag protein on the cell surface with Alexa Fluor 555–DBCO **4n**.
HEK293 cells expressing HaloTag on the surface were incubated with (+) or without (–) azido-HaloTag ligand **2b**, followed by with Alexa Fluor 555–DBCO **4n** or Alexa Fluor 555 azide (**2i**). Vector (+) indicates the expression of the HaloTag fusion proteins, and vector (–) indicates no expression. Scale bar, 5 μm.

Characterization Data of New Compounds

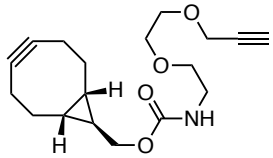
4-(11,12-Didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yloxycarbonylaminomethyl)-1-(4-(methoxycarbonyl)benzyl)-1*H*-1,2,3-triazole (**4a**)[S8] was identical in the spectra data with those reported in the literature.

(1α,8α,9α)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-(2-propyn-1-yl)carbamate (**1b**)



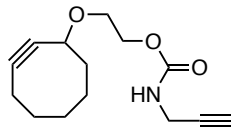
Colorless solid; Mp 75–80 °C; R_f = 0.39 (*n*-hexane/EtOAc = 2/1); ^{1}H NMR (500 MHz, CD_3OD) δ 0.64–0.83 (m, 3H), 1.30–1.45 (m, 2H), 2.05–2.15 (m, 2H), 2.19–2.30 (m, 2H), 2.34–2.44 (m, 2H), 2.57 (t, 1H, *J* = 2.4 Hz), 3.86 (d, 2H, *J* = 2.4 Hz), 3.97 (d, 2H, *J* = 7.0 Hz); ^{13}C NMR (126 MHz, CD_3OD) δ 21.9 (2C), 24.2 (2C), 25.0 (1C), 30.9 (1C), 34.4 (2C), 70.3 (1C), 71.9 (1C), 81.3 (1C), 99.4 (2C), 158.9 (1C); IR (KBr, cm^{-1}) 655, 988, 1247, 1519, 1710, 2122, 2188, 2265, 3300; HRMS (ESI^+) *m*/*z* 254.1149 ($[M+Na]^+$, $C_{14}H_{17}NNaO_2^+$ requires 254.1151).

(1α,8α,9α)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-(2-(2-(2-propyn-1-yloxy)ethoxy)ethyl)carbamate (**1c**)



Pale yellow oil; TLC R_f 0.22 (*n*-hexane/EtOAc = 2/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.63–0.79 (m, 3H), 1.30–1.44 (m, 2H), 2.11–2.20 (m, 2H), 2.23–2.34 (m, 2H), 2.37–2.44 (m, 2H), 2.45 (t, 1H, *J* = 2.4 Hz), 3.39 (dt, 2H, *J* = 5.1, 5.1 Hz), 3.57 (t, 2H, *J* = 5.1 Hz), 3.63–3.67 (m, 2H), 3.67–3.72 (m, 2H), 3.98 (d, 2H, *J* = 6.9 Hz), 4.21 (d, 2H, *J* = 2.4 Hz), 5.13 (br, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.4 (2C), 22.8 (2C), 23.7 (1C), 33.3 (2C), 40.7 (1C), 58.4 (1C), 69.0 (1C+1C, two signals overlapped), 70.1 (1C), 70.2 (1C), 74.7 (1C), 79.5 (1C), 98.8 (2C), 156.8 (1C); IR (KBr, cm^{-1}); 1025, 1096, 1134, 1522, 1714, 2114, 2915, 3289, 3347; HRMS (ESI^+) *m*/*z* 342.1671 ($[M+Na]^+$, $C_{18}H_{25}NNaO_4^+$ requires 342.1676).

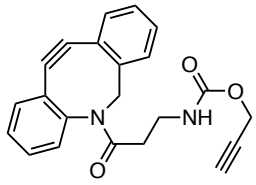
2-(Cyclooctyn-3-yloxy)ethyl *N*-(2-propyn-1-yl)carbamate (**1d**)



Pale yellow oil; TLC R_f 0.22 (*n*-hexane/EtOAc = 4/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39–1.49 (m, 1H), 1.56–1.75 (m, 2H), 1.78–1.88 (m, 2H), 1.90–2.02 (m, 2H), 2.09–2.21 (m, 2H), 2.21–2.30 (m, 1H), 2.24 (t, 1H, *J* = 2.5 Hz), 3.50–3.60 (m, 1H), 3.76 (ddd, 1H, *J* = 11.5, 6.1, 3.1 Hz), 3.98 (dd, 2H, *J* = 5.7, 2.5 Hz), 4.17–4.26 (m, 2H), 4.29 (ddd, 1H, *J* = 11.5, 6.1, 3.1 Hz), 4.97 (br, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 20.7 (1C), 26.3 (1C), 29.7 (1C), 30.8 (1C), 34.3 (1C), 42.2 (1C), 64.3 (1C), 67.3 (1C), 71.6 (1C), 72.7 (1C),

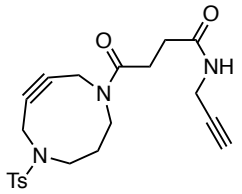
79.6 (1C), 92.4 (1C), 100.4 (1C), 155.9 (1C); IR (KBr, cm^{-1}); 1043, 1103, 1248, 1339, 1450, 1524, 1717, 2930, 3304; HRMS (ESI^+) *m/z* 272.1256 ($[M+Na]^+$, $C_{14}H_{19}NNaO_3^+$ requires 272.1257).

2-Propynyl *N*-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropyl)carbamate (**1e**)



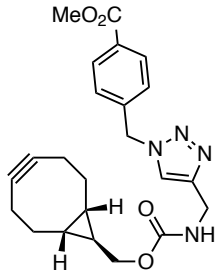
Colorless solid; Mp 115–117 °C; TLC R_f 0.47 (*n*-hexane/EtOAc = 1/2); ^1H NMR (500 MHz, $CDCl_3$) δ 1.96 (ddd, 1H, *J* = 16.7, 6.5, 4.4 Hz), 2.47 (dd, 1H, *J* = 2.2, 2.2 Hz), 2.54 (ddd, 1H, *J* = 16.7, 7.7, 4.7 Hz), 3.18–3.34 (m, 2H), 3.71 (d, 1H, *J* = 13.9 Hz), 4.60 (dd, 1H, *J* = 15.7, 2.2 Hz), 4.65 (dd, 1H, *J* = 15.7, 2.2 Hz), 5.15 (d, 1H, *J* = 13.9 Hz), 5.38 (br, 1H), 7.27–7.36 (m, 3H), 7.37–7.45 (m, 4H), 7.66–7.70 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 34.7 (1C), 37.0 (1C), 52.2 (1C), 55.5 (1C), 74.3 and 74.4 (1C, duplicate signals derived from rotamers), 78.4 (1C), 107.4 (1C), 114.9 (1C), 122.6 (1C), 122.8 (1C), 125.6 (1C), 127.1 (1C), 127.8 (1C), 128.27 (1C), 128.31 (1C), 128.5 (1C), 129.0 (1C), 132.0 (1C), 147.9 (1C), 151.0 (1C), 155.3 (1C), 171.8 (1C); IR (KBr, cm^{-1}) 754, 1233, 1250, 1290, 1398, 1435, 1447, 1481, 1514, 1651, 1724; HRMS (ESI^+) *m/z* 381.1203 ($[M+Na]^+$, $C_{22}H_{18}N_2NaO_3^+$ requires 381.1210).

N-(2-Propyn-1-yl) 3-(4-tosyl-4,8-diazacyclononyn-8-ylcarbonyl)propionamide (**1f**)



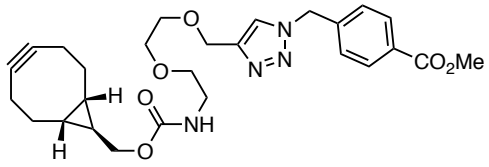
Colorless solid; Mp 134–136 °C; TLC R_f 0.18 (*n*-hexane/ EtOAc = 1/6); ^1H NMR (500 MHz, $CDCl_3$) δ 2.08–2.15 (m, 2H), 2.18 (t, 0.4H, *J* = 2.5 Hz), 2.21 (t, 0.6H, *J* = 2.5 Hz), 2.436 (s, 0.4H × 3), 2.442 (s, 0.6H × 3), 2.52 (t, 0.4H × 2, *J* = 6.1 Hz), 2.56 (t, 0.6H × 2, *J* = 6.3 Hz), 2.62–2.69 (m, 2H), 3.15–3.18 (m, 0.4H × 2), 3.25–3.29 (m, 0.6H × 2), 3.56–3.59 (m, 0.4H × 2), 3.60–3.63 (m, 0.6H × 2), 3.84–3.88 (m, 2H), 3.99–4.03 (m, 2H), 4.05 (t, 0.4H × 2, *J* = 2.5 Hz), 4.21 (t, 0.6H × 2, *J* = 2.5 Hz), 6.15 (br, 0.6H), 6.21 (br, 0.4H), 7.31–7.35 (m, 2H), 7.64–7.69 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.52 (0.4C), 21.53 (0.6C), 29.0 (0.6C), 29.15 (0.4C), 29.18 (0.4C), 29.20 (0.6C), 30.0 (0.4C), 31.0 (0.4C), 31.1 (0.6C), 32.1 (0.6C), 36.7 (0.6C), 39.4 (0.4C), 40.7 (0.6C), 40.9 (0.4C), 43.7 (0.6C), 43.8 (0.4C), 45.3 (0.6C+0.4C, two signals overlapped), 71.4 (0.4C), 71.5 (0.6C), 79.50 (0.6C), 79.52 (0.4C), 86.8 (0.6C), 88.2 (0.4C), 88.4 (0.4C), 89.0 (0.6C), 127.28 (0.6C × 2), 127.33 (0.4C × 2), 129.88 (0.4C × 2), 129.93 (0.6C × 2), 134.47 (0.4C), 134.55 (0.6C), 143.8 (0.4C), 143.9 (0.6C), 171.2 (0.4C), 171.7 (0.6C), 171.8 (0.4C), 172.4 (0.6C); IR (KBr, cm^{-1}) 1160, 1336, 1420, 1449, 1534, 1643, 2927, 3295; HRMS (ESI^+) *m/z* 438.1451 ($[M+Na]^+$, $C_{21}H_{25}N_3NaO_4S^+$ requires 438.1458).

4-((1α,8α,9α)-Bicyclo[6.1.0]non-4-yn-9-ylmethoxycarbonylaminomethyl)-1-(4-(methoxycarbonyl)-benzyl)-1*H*-1,2,3-triazole (**4b**)



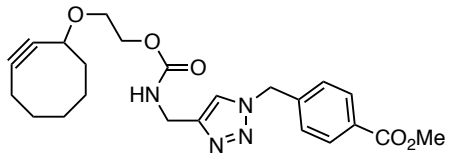
Colorless solid; Mp 137–139 °C; TLC R_f 0.19 (CH_2Cl_2/MeOH = 30/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.60–0.76 (m, 3H), 1.28–1.41 (m, 2H), 2.10–2.18 (m, 2H), 2.21–2.32 (m, 2H), 2.32–2.41 (m, 2H), 3.92 (s, 3H), 3.96 (d, 2H, J = 6.9 Hz), 4.43 (d, 2H, J = 6.0 Hz), 5.27 (br, 1H), 5.56 (s, 2H), 7.29–7.33 (AA'BB', 2H), 7.48 (s, 1H), 8.01–8.06 (AA'BB', 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.3 (2C), 22.8 (2C), 23.6 (1C), 33.2 (2C), 36.4 (1C), 52.3 (1C), 53.7 (1C), 69.4 (1C), 98.7 (2C), 122.0 (1C), 127.8 (2C), 130.4 (2C), 130.6 (1C), 139.3 (1C), 145.8 (1C), 156.7 (1C), 166.3 (1C); IR (KBr, cm^{-1}) 733, 1112, 1280, 1436, 1522, 1716, 2930, 3359; HRMS (ESI$^+$) *m*/*z* 445.1833 ([M+Na]$^+$, $C_{23}H_{26}N_4NaO_4^+$ requires 445.1846).

4-((2-(2-((1α,8α,9α)-Bicyclo[6.1.0]non-4-yn-9-ylmethoxycarbonylamino)ethoxy)ethoxy)methyl)-1-(4-(methoxycarbonyl)benzyl)-1*H*-1,2,3-triazole (**4c**)



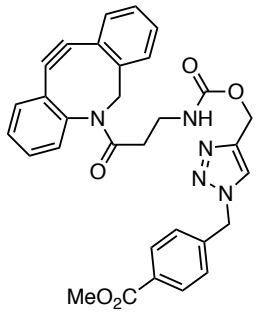
Colorless solid; Mp 111–120 °C; TLC R_f 0.18 (CH_2Cl_2/MeOH = 30/1); ^{1}H NMR (500 MHz, $CDCl_3$,) δ 0.60–0.78 (m, 3H), 1.29–1.42 (m, 2H), 2.10–2.19 (m, 2H), 2.23–2.32 (m, 2H), 2.35–2.43 (m, 2H), 3.32–3.39 (m, 2H), 3.53 (t, 2H, J = 5.2 Hz), 3.59–3.65 (m, 2H), 3.65–3.71 (m, 2H), 3.92 (s, 3H), 3.96 (d, 2H, J = 6.9 Hz), 4.68 (s, 2H), 5.16 (br, 1H), 5.59 (s, 2H), 7.30–7.35 (AA'BB', 2H), 7.52 (s, 1H), 8.01–8.07 (AA'BB', 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.4 (2C), 22.8 (2C), 23.7 (1C), 33.3 (2C), 40.7 (1C), 52.3 (1C), 53.7 (1C), 64.6 (1C), 69.0 (1C), 69.7 (1C), 70.17 (1C), 70.25 (1C), 98.8 (2C), 122.6 (1C), 127.8 (2C), 130.4 (2C), 130.6 (1C), 139.4 (1C), 145.7 (1C), 156.8 (1C), 166.4 (1C); IR (KBr, cm^{-1}) 733, 1111, 1280, 1436, 1522, 1716, 2927, 3325; HRMS (ESI$^+$) *m*/*z* 533.2371 ([M+Na]$^+$, $C_{27}H_{34}N_4NaO_6^+$ requires 533.2371).

4-(2-(Cyclooctyn-3-yloxy)ethoxycarbonylaminomethyl)-1-(4-(methoxycarbonyl)benzyl)-1*H*-1,2,3-triazole (**4d**)



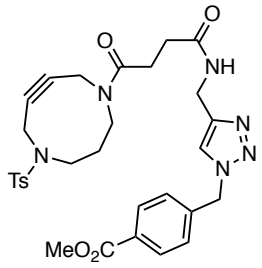
Colorless solid; Mp 66–70 °C; TLC R_f 0.21 (CH_2Cl_2/MeOH = 30/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37–1.47 (m, 1H), 1.54–1.73 (m, 2H), 1.76–1.87 (m, 2H), 1.88–1.99 (m, 2H), 2.05–2.19 (m, 2H), 2.19–2.29 (m, 1H), 3.47–3.57 (m, 1H), 3.73 (ddd, 1H, J = 11.2, 6.2, 3.2 Hz), 3.92 (s, 3H), 4.13–4.27 (m, 3H), 4.44 (d, 2H, J = 6.1 Hz), 5.32 (br, 1H), 5.56 (s, 2H), 7.28–7.34 (AA'BB', 2H), 7.49 (s, 1H), 8.02–8.06 (AA'BB', 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 20.7 (1C), 26.3 (1C), 29.7 (1C), 34.2 (1C), 36.5 (1C), 42.2 (1C), 52.3 (1C), 53.7 (1C), 64.2 (1C), 67.3 (1C), 72.7 (1C), 92.3 (1C), 100.4 (1C), 122.1 (1C), 127.8 (2C), 130.4 (2C), 130.6 (1C), 139.3 (1C), 145.8 (1C) 156.4 (1C), 166.4 (1C); IR (KBr, cm^{-1}) 737, 1108, 1280, 1435, 1527, 1722, 2929, 3341; HRMS (ESI^+) m/z 463.1965 ($[M+Na]^+$, $C_{23}H_{28}N_4NaO_5^+$ requires 463.1952).

4-(3-(5*H*,6*H*-11,12-Didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropylaminocarbonyloxymethyl)-1-(4-(methoxycarbonyl)benzyl)-1*H*-1,2,3-triazole (**4e**)



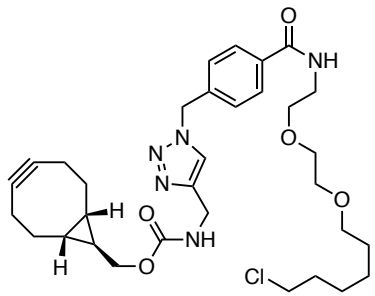
Colorless solid; Mp 174–176 °C; TLC R_f 0.58 (CH_2Cl_2/MeOH = 9/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.93 (ddd, 1H, J = 16.7, 6.7, 4.0 Hz), 2.48 (ddd, 1H, J = 16.7, 7.8, 4.3 Hz), 3.11–3.26 (m, 2H), 3.68 (d, 1H, J = 13.9 Hz), 3.92 (s, 3H), 5.11 (d, 1H, J = 13.9 Hz), 5.12 (s, 2H), 5.27 (dd, 1H, J = 5.9, 5.9 Hz), 5.57 (s, 2H), 7.19–7.23 (m, 1H), 7.26–7.41 (m, 8H), 7.52 (s, 1H), 7.61–7.66 (m, 1H), 8.04 (d, 2H, J = 8.3 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 34.8 (1C), 36.9 (1C), 52.3 (1C), 53.7 (1C), 55.5 (1C), 57.9 (1C), 107.4 (1C), 115.0 (1C), 122.7 (1C), 122.8 (1C), 123.5 (1C), 125.7 (1C), 127.2 (1C), 127.8 (1C), 127.9 (2C), 128.3 (1C), 128.4 (1C), 128.5 (1C), 129.1 (1C), 130.4 (2C), 130.6 (1C), 132.0 (1C), 139.3 (1C), 144.3 and 144.4 (1C, duplicate signals derived from rotamers), 147.9 (1C), 151.0 (1C), 155.9 (1C), 166.4 (1C), 171.8 (1C); IR (KBr, cm^{-1}) 741, 754, 1229, 1252, 1281, 1437, 1647, 1719, 3414; HRMS (ESI^+) m/z 572.1886 ($[M+Na]^+$, $C_{31}H_{27}N_5NaO_5^+$ requires 572.1904).

1-(4-(Methoxycarbonyl)benzyl)-4-(3-(4-tosyl-4,8-diazacyclononyn-8-ylcarbonyl)propionyl-aminomethyl)-1*H*-1,2,3-triazole (**4f**)



Colorless solid; Mp 137–139 °C; TLC R_f 0.25 (CH_2Cl_2/MeOH = 15/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.05–2.13 (m, 2H), 2.43 (s, 0.4H × 3), 2.44 (s, 0.6H × 3), 2.48 (t, 0.4H × 2, *J* = 6.3 Hz), 2.52 (t, 0.6H × 2, *J* = 6.3 Hz), 2.60–2.67 (m, 2H), 3.15 (t, 0.4H × 2, *J* = 5.5 Hz), 3.26 (t, 0.6H × 2, *J* = 5.6 Hz), 3.54 (t, 0.4H × 2, *J* = 5.5 Hz), 3.59 (t, 0.6H × 2, *J* = 5.6 Hz), 3.83–3.87 (m, 2H), 3.917 (s, 0.6H × 3), 3.922 (s, 0.4H × 3), 4.03 (t, 0.4H × 2, *J* = 2.3 Hz), 4.15 (t, 0.6H × 2, *J* = 2.5 Hz), 4.47–4.50 (m, 2H), 5.54 (s, 2H), 6.46–6.52 (m, 1H), 7.29–7.35 (m, 4H), 7.50 (s, 0.6H), 7.51 (s, 0.4H), 7.64–7.68 (m, 2H), 8.01–8.05 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.51 (0.4C), 21.53 (0.6C), 28.99 (0.6C), 29.05 (0.4C), 30.1 (0.4C), 30.9 (0.4C), 31.1 (0.6C), 32.1 (0.6C), 35.13 (0.6C), 35.15 (0.4C), 36.6 (0.6C), 39.3 (0.4C), 40.7 (0.6C), 40.9 (0.4C), 43.7 (0.6C), 43.8 (0.4C), 45.3 (0.6C+0.4C, two signals overlapped), 52.3 (0.6C+0.4C, two signals overlapped), 53.7 (0.6C+0.4C, two signals overlapped), 86.8 (0.6C), 88.2 (0.4C), 88.4 (0.4C), 89.0 (0.6C), 122.18 (0.6C), 122.21 (0.4C), 127.26 (0.6C × 2), 127.32 (0.4C × 2), 127.81 (0.4C × 2), 127.85 (0.6C × 2), 129.88 (0.4C × 2), 129.93 (0.6C × 2), 130.4 (0.6C × 2+0.4C × 3, three signals overlapped), 130.6 (0.6C), 134.45 (0.4C), 134.53 (0.6C), 139.3 (0.6C), 139.4 (0.4C), 143.8 (0.4C), 143.9 (0.6C), 145.57 (0.6C), 145.58 (0.4C), 166.36 (0.6C), 166.38 (0.4C), 171.2 (0.4C), 172.16 (0.6C), 172.19 (0.4C), 172.4 (0.6C); IR (KBr, cm^{-1}) 551, 587, 716, 812, 1112, 1158, 1283, 1334, 1418, 1446, 1537, 1634, 1723, 2933, 3320; HRMS (ESI^+) *m*/*z* 629.2143 ($[M+Na]^+$, $C_{30}H_{34}N_6NaO_6S^+$ requires 629.2153).

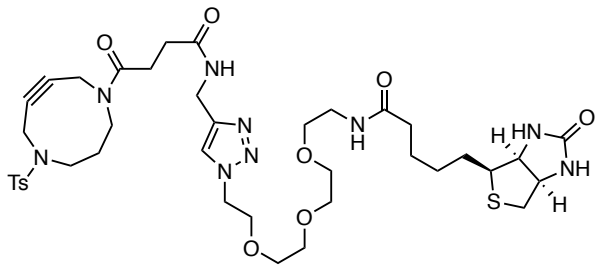
4-((1α,8α,9α)-Bicyclo[6.1.0]non-4-yn-9-ylmethoxycarbonylaminomethyl)-1-(4-(2-(2-(6-chlorohexyl-oxy)ethoxy)ethylaminocarbonyl)benzyl)1*H*-1,2,3-triazole (**4g**)



Colorless solid; Mp 91–93 °C; TLC R_f 0.48 (CH_2Cl_2/MeOH = 9/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.62–0.75 (m, 3H), 1.31–1.45 (m, 6H), 1.54–1.59 (m, 2H), 1.72–1.77 (m, 2H), 2.09–2.18 (m, 2H), 2.22–2.31 (m, 2H), 2.36–2.40 (m, 2H), 3.46 (t, 2H, *J* = 6.7 Hz), 3.52 (t, 2H, *J* = 6.7 Hz), 3.57–3.60 (m, 2H), 3.63–

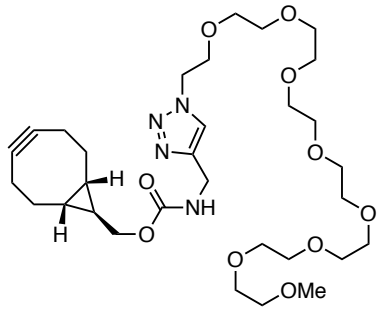
3.69 (m, 6H), 3.96 (d, 2H, J = 6.9 Hz), 4.43 (d, 2H, J = 6.0 Hz), 5.21–5.28 (br, 1H), 5.55 (s, 2H), 6.69–6.75 (br, 1H), 7.31 (d, 2H, J = 8.3 Hz), 7.47 (s, 1H), 7.79 (d, 2H, J = 8.3 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.4 (2C), 22.8 (2C), 23.6 (1C), 25.4 (1C), 26.6 (1C), 29.4 (1C), 32.4 (1C), 33.2 (2C), 36.4 (1C), 39.7 (1C), 45.0 (1C), 53.7 (1C), 69.4 (1C), 69.7 (1C), 70.0 (1C), 70.2 (1C), 71.2 (1C), 98.8 (2C), 122.0 (1C), 127.8 (2C), 128.0 (2C), 135.1 (1C), 137.8 (1C), 145.7 (1C), 156.7 (1C), 166.5 (1C); IR (KBr, cm^{-1}) 737, 1049, 1098, 1123, 1258, 1308, 1618, 1641, 1701, 2932, 3387; HRMS (ESI^+) m/z 636.2901 ($[M+Na]^+$, $C_{32}H_{44}{}^{35}ClN_5NaO_5^+$ requires 636.2923).

1-(2-(2-(2-(2-(Biotinamido)ethoxy)ethoxy)ethoxy)ethyl)-4-(3-(4-tosyl-4,8-diazacyclononyn-8-yl-carbonyl)propionylaminomethyl)-1*H*-1,2,3-triazole (**4h**)



Colorless solid; Mp 66–68 °C; TLC R_f 0.29 (CH_2Cl_2/MeOH = 9/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38–1.47 (m, 2H), 1.54–1.77 (m, 4H), 2.03–2.18 (m, 4H), 2.19–2.33 (m, 2H), 2.43 (s, 3H), 2.49–2.75 (m, 6H), 2.87–2.93 (m, 1H), 3.11–3.18 (m, 2H), 3.24–3.29 (m, 1H), 3.37–3.44 (m, 2H), 3.51–3.65 (m, 10H), 3.81–3.91 (m, 4H), 4.04–4.09 (br, 1H), 4.16–4.21 (br, 1H), 4.30–4.35 (m, 1H), 4.41–4.54 (m, 4H), 5.53–5.61 (br, 1H), 6.56–6.61 (br, 1H), 6.97–7.03 (br, 1H), 7.31–7.37 (AA'BB', 2H), 7.59–7.68 (m, 3H), 7.76 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.5, 25.4, 28.1, 28.2, 29.0, 30.1, 30.7, 30.9, 32.1, 34.8, 35.6, 36.6, 39.1, 39.4, 40.5, 40.6, 40.9, 43.8, 45.35, 45.42, 50.2, 55.7, 60.1, 61.9, 69.3, 69.9, 70.0, 70.3, 70.5, 86.8, 88.2, 88.4, 89.1, 123.3, 127.2, 127.3, 129.88, 129.92, 134.4, 134.5, 143.8, 143.9, 144.9, 163.9, 171.5, 172.31, 172.34, 172.6, 173.4: Analysis of rotamers was not conducted; IR (KBr, cm^{-1}) 415, 540, 584, 654, 712, 741, 1069, 1096, 1111, 1159, 1265, 1333, 1427, 1452, 1535, 1547, 1643, 1692, 2926, 3291; HRMS (ESI^+) m/z 882.3588 ($[M+Na]^+$, $C_{39}H_{57}N_9NaO_9S_2^+$ requires 882.3613).

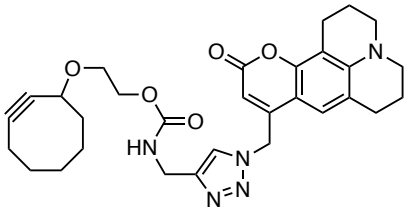
4-((1α,8α,9α)-Bicyclo[6.1.0]non-4-yn-9-ylmethoxycarbonylaminomethyl)-1-(2-(2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazole (**4i**)



Colorless oil; TLC R_f 0.16 (CH_2Cl_2/MeOH = 15/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.63–0.76 (m, 3H),

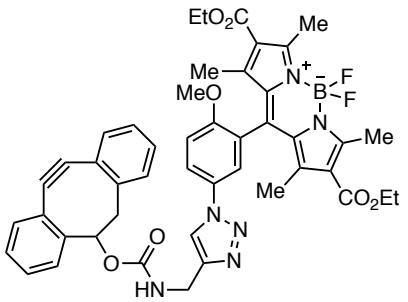
1.31–1.42 (m, 2H), 2.11–2.19 (m, 2H), 2.23–2.33 (m, 2H), 2.35–2.43 (m, 2H), 3.38 (s, 3H), 3.55–3.57 (m, 2H), 3.60–3.67 (m, 26H), 3.86 (t, 2H, J = 5.1 Hz), 3.98 (d, 2H, J = 6.8 Hz), 4.45 (d, 2H, J = 5.9 Hz), 4.52 (t, 2H, J = 5.1 Hz), 5.41 (br, 1H), 7.72 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.3 (2C), 22.8 (2C), 23.6 (1C), 33.2 (2C), 36.5 (1C), 50.2 (1C), 59.0 (1C), 69.2 (1C), 69.4 (1C), 70.4–70.5 (m, 13C), 71.9 (1C), 98.7 (2C), 123.0 (1C), 144.8 (1C), 156.7 (1C); IR (KBr, cm^{-1}) 739, 913, 1106, 1251, 1452, 1524, 1716, 2900, 3327; HRMS (ESI^+) m/z 663.3554 ($[M+Na]^+$, $C_{31}H_{52}N_4NaO_{10}^+$ requires 663.3576).

4-(2-(Cyclooctyn-3-yloxy)ethoxycarbonylaminomethyl)-1-((11-oxo-2,3,6,7-tetrahydro-1*H*,5*H*,11*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-9-yl)methyl)-1*H*-1,2,3-triazole (**4j**)



Pale yellow solid; Mp 155 °C (decomposition); TLC R_f 0.32 (CH_2Cl_2/MeOH = 20/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.38–1.48 (m, 1H), 1.54–1.74 (m, 2H), 1.75–1.88 (m, 2H), 1.88–2.01 (m, 6H), 2.05–2.31 (m, 3H), 2.73 (t, 2H, J = 6.3 Hz), 2.87 (t, 2H, J = 6.5 Hz), 3.26 (t, 2H, J = 7.0 Hz), 3.27 (t, 2H, J = 7.0 Hz), 3.46–3.60 (m, 1H), 3.69–3.79 (m, 1H), 4.17–4.29 (m, 3H), 4.44 (d, 2H, J = 6.1 Hz), 5.31 (br, 1H), 5.54 (s, 2H), 5.67 (s, 1H), 6.96 (s, 1H), 7.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 20.3 (1C), 20.4 (1C), 20.6 (1C), 21.3 (1C), 26.3 (1C), 27.7 (1C), 29.7 (1C), 34.2 (1C), 36.5 (1C), 42.2 (1C), 49.5 (1C), 49.9 (1C), 50.4 (1C), 64.2 (1C), 67.3 (1C), 72.7 (1C), 92.4 (1C), 100.4 (1C), 105.7 (1C), 107.0 (1C), 107.5 (1C), 118.6 (1C), 120.5 (1C), 122.4 (1C), 145.9 (1C), 146.4 (1C), 147.9 (1C), 151.4 (1C), 156.3 (1C), 161.6 (1C); IR (KBr, cm^{-1}) 729, 913, 1047, 1181, 1255, 1314, 1439, 1524, 1601, 1712; HRMS (ESI^+) m/z 568.2529 ($[M+Na]^+$, $C_{30}H_{35}N_5NaO_5^+$ requires 568.2530).

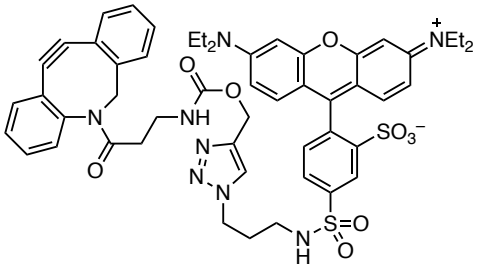
1-(3-(2,6-Bis(ethoxycarbonyl)-4,4-difluoro-1,3,5,7-tetramethyl-3a,4a-diaza-4-bora-*s*-indacen-8-yl)-4-methoxyphenyl)-4-(11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yloxycarbonylamino-methyl)-1*H*-1,2,3-triazole (**4k**)



Red solid; Mp 140–142 °C; TLC R_f 0.68 (CH_2Cl_2/MeOH = 9/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (t, 6H, J = 7.1 Hz), 1.76–1.82 (m, 6H), 2.84 (s, 6H), 2.88 (dd, 1H, J = 15.2, 3.8 Hz), 3.15 (dd, 1H, J = 15.2,

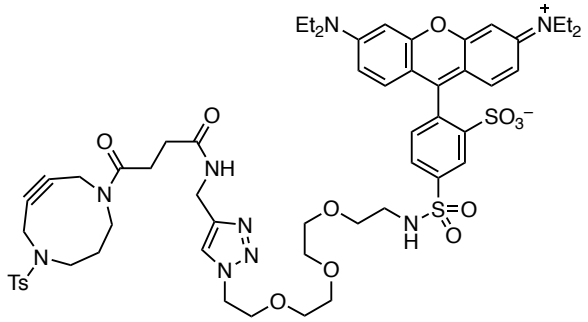
1.9 Hz), 3.85 (s, 3H), 4.27 (q, 4H, J = 7.1 Hz), 4.49 (dd, 1H, J = 15.5, 6.0 Hz), 4.55 (dd, 1H, J = 15.5, 6.0 Hz), 5.47–5.52 (br, 1H), 5.71–5.77 (m, 1H), 7.14 (d, 1H, J = 8.9 Hz), 7.20–7.33 (m, 7H), 7.43 (d, 1H, J = 7.5 Hz), 7.54 (d, 1H, J = 2.5 Hz), 7.84 (dd, 1H, J = 8.9, 2.5 Hz), 7.89 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 13.2 (2C), 14.3 (2C), 15.1 (2C), 36.4 (1C), 46.0 (1C), 56.3 (1C), 60.3 (2C), 77.2 (1C), 109.9 (1C), 112.2 (1C), 112.9 (1C), 120.4 (1C), 121.2 (1C), 121.7 (2C), 122.5 (1C), 123.3 (1C), 123.6 (1C), 123.8 (1C), 124.6 (1C), 126.0 (1C), 126.3 (1C), 127.09 (1C), 127.12 (1C), 127.8 (1C), 128.0 (1C), 129.8 (1C), 131.2 (2C), 131.5 (1C), 140.4 (1C), 145.9 (1C), 146.7 (2C), 150.8 (1C), 151.7 (1C), 155.5 (1C), 156.5 (1C), 159.7 (d, 2C, J = 4.2 Hz), 164.2 (2C); ^{19}F NMR (376 Hz, $CDCl_3$) δ –143.5 to –142.9 (m); IR (KBr, cm^{-1}) 1009, 1036, 1109, 1184, 1256, 1314, 1508, 1528, 1707; HRMS (ESI$^+$) m/z 863.3139 ([M+Na]$^+$, $C_{46}H_{43}BF_2N_6NaO_7^+$ requires 863.3154).

1-(3-(4-(3,6-Bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido)propyl)-4-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropylaminocarbonyloxymethyl)-1*H*-1,2,3-triazole (**4l**)



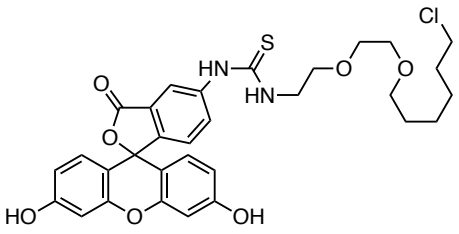
Purple solid; Mp 266–268 °C; TLC R_f 0.45 (CH_2Cl_2/MeOH = 9/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28 (t, 12H, J = 7.0 Hz), 1.87–1.96 (m, 1H), 2.00–2.08 (m, 2H), 2.47–2.55 (m, 1H), 2.96–3.06 (m, 2H), 3.09–3.18 (m, 2H), 3.45–3.69 (m, 9H), 4.33 (t, 2H, J = 6.8 Hz), 4.98 (d, 1H, J = 12.7 Hz), 5.05 (d, 1H, J = 12.7 Hz), 5.10 (d, 1H, J = 15.4 Hz), 5.48 (t, 1H, J = 5.7 Hz), 6.61 (d, 2H, J = 1.4 Hz), 6.83 (t, 1H, J = 5.6 Hz), 6.86–6.92 (m, 2H), 7.18–7.22 (m, 2H), 7.26–7.38 (m, 8H), 7.61–7.66 (m, 1H), 7.71 (s, 1H), 7.95 (d, 1H, J = 7.5 Hz), 8.78 (d, 1H, J = 1.3 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 12.6 (4C), 30.2 (1C), 34.9 (1C), 36.9 (1C), 40.1 (1C), 45.8 (4C), 47.2 (1C), 55.4 (1C), 58.2 (1C), 95.4 (2C), 107.7 (1C), 113.9 (2C), 114.3, 114.7 (1C), 122.3 (1C), 123.0 (1C), 124.2, 125.6 (1C), 127.0 (1C), 127.2 (1C), 127.5, 127.7 (1C), 128.2 (1C), 128.3 (1C), 128.8 (1C), 129.3, 129.6, 132.0 (1C), 133.49 (2C), 133.55, 141.7 (1C), 143.3 (1C), 147.4 (1C), 148.0 (1C), 151.0 (1C), 155.5 (1C), 156.0 (1C), 157.7 , 158.5 (1C), 171.7 (1C): three pairs of equivalent carbons (2C) were not identified; IR (KBr, cm^{-1}) 683, 1076, 1134, 1180, 1198, 1246, 1275, 1339, 1416, 1466, 1481, 1591, 1647, 3433; HRMS (ESI$^+$) m/z 1021.3378 ([M+Na]$^+$, $C_{52}H_{54}N_8NaO_9S_2^+$ requires 1021.3347).

1-(2-(2-(2-(2-(4-(3,6-Bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido)ethoxy)-ethoxy)ethoxy)ethyl)-4-(3-(4-tosyl-4,8-diazacyclononyn-8-ylcarbonyl)propionylaminomethyl)1*H*-1,2,3-triazole (**4m**)



Purple solid; Mp 210–215 °C; TLC R_f 0.59 (CH_2Cl_2/MeOH = 6/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27–1.34 (m, 12H), 2.01–2.11 (m, 2H), 2.42 (s, 3H), 2.46 (t, 0.4H × 2, J = 7.2 Hz), 2.53–2.58 (m, 0.6H × 2), 2.59–2.65 (m, 2H), 3.13–3.17 (m, 0.4H × 2), 3.21–3.32 (m, 2H+0.6H × 2), 3.46–3.69 (m, 20H), 3.80–3.82 (m, 0.4H × 2), 3.84–3.86 (m, 0.6H × 2), 3.88–3.92 (m, 2H), 4.01–4.04 (m, 0.4H × 2), 4.16–4.20 (m, 0.6H × 2), 4.42–4.50 (m, 4H), 6.66–6.69 (m, 2H), 6.80–6.83 (m, 2H), 7.02–7.08 (m, 1H), 7.17 (d, 0.4H × 2, J = 9.5 Hz), 7.19 (d, 0.6H × 2, J = 9.5 Hz), 7.200 (d, 0.4H, J = 7.9 Hz), 7.203 (d, 0.6H, J = 7.9 Hz), 7.30–7.35 (m, 2H), 7.43–7.49 (m, 1H), 7.63–7.67 (m, 2H), 7.93 (s, 0.6H), 7.96 (s, 0.4H), 7.99–8.02 (m, 1H), 8.82 (d, 0.4H, J = 1.8 Hz), 8.83 (d, 0.6H, J = 1.8 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 12.6, 20.9, 21.5, 29.2, 29.5, 30.1, 31.06, 31.12, 35.3, 36.6, 37.4, 39.5, 40.6, 41.0, 43.1, 43.6, 43.7, 45.3, 45.4, 45.8, 50.2, 69.4, 69.5, 70.1, 70.50, 70.52, 70.6, 86.6, 87.6, 89.6, 95.6, 113.6, 114.28, 114.31, 123.9, 124.0, 126.9, 127.1, 127.3, 129.6, 129.88, 129.90, 133.28, 133.32, 133.5, 134.5, 134.7, 142.3, 142.4, 143.7, 143.8, 145.1, 145.2, 148.1, 148.2, 155.5, 157.8, 158.8, 158.9, 171.8, 172.49, 172.52, 172.9: Analysis of rotamers was not conducted; IR (KBr, cm^{-1}) 549, 744, 913, 1181, 1338, 1591, 2934; HRMS (ESI$^+$) m/z 1196.4245 ([M+Na]$^+$, $C_{56}H_{71}N_9NaO_{13}S_3^+$ requires 1196.4226).

1-(2-(2-(6-Chlorohexyloxy)ethoxy)ethyl)-3-(4-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-3-carboxyphenyl)-thiourea (**5**)



Yellow solid; Mp 186–188 °C; TLC R_f 0.38 (CH_2Cl_2/MeOH = 9/1); ^{1}H NMR (500 MHz, CD_3OD) δ 1.27–1.48 (m, 4H), 1.55 (tt, 2H, J = 7.0, 7.0 Hz), 1.72 (tt, 2H, J = 7.0, 7.0 Hz), 3.46 (t, 2H, J = 6.6 Hz), 3.51 (t, 2H, J = 6.6 Hz), 3.58–3.63 (m, 2H), 3.63–3.68 (m, 2H), 3.68–3.73 (m, 2H), 3.77–3.87 (br, 2H), 6.54 (dd, 2H, J = 8.7, 1.7 Hz), 6.65–6.69 (m, 4H), 7.15 (d, 1H, J = 8.0 Hz), 7.78 (d, 1H, J = 8.0 Hz), 8.16 (s, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ 23.5 (1C), 24.8 (1C), 27.6 (1C), 30.8 (1C), 42.6 (1C), 42.7

(1C), 67.3 (1C), 68.2 (1C), 68.4 (1C), 69.3 (1C), 100.6, 108.5, 110.6, 116.6, 122.7, 127.4, 151.2, 158.4, 168.2: Some signals for aromatic carbons were not detected; IR (KBr, cm^{-1}) 851, 1113, 1179, 1258, 1456, 1607, 1734, 2930, 3271; HRMS (ESI^+) *m*/*z* 613.1744 ($[M+H]^+$, $C_{31}H_{34}ClN_2O_7S^+$ requires 613.1770).

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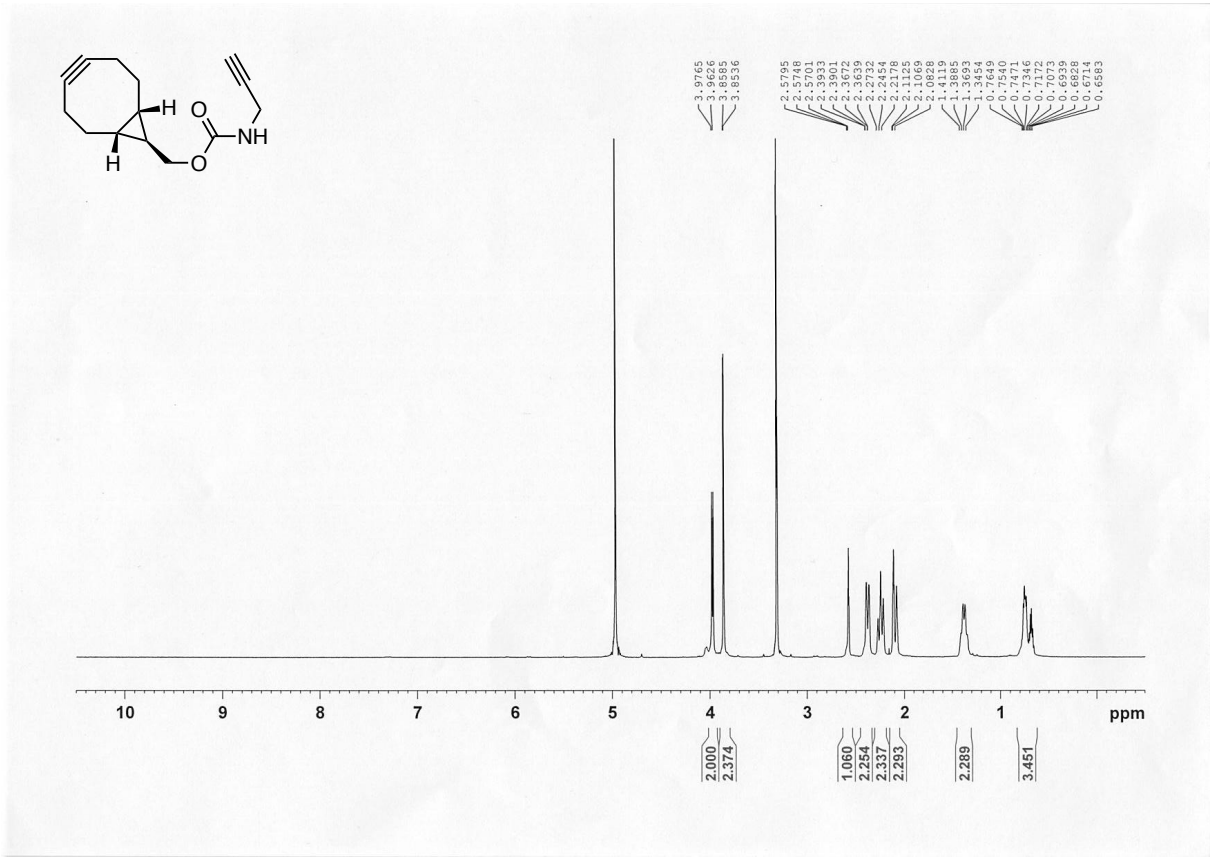
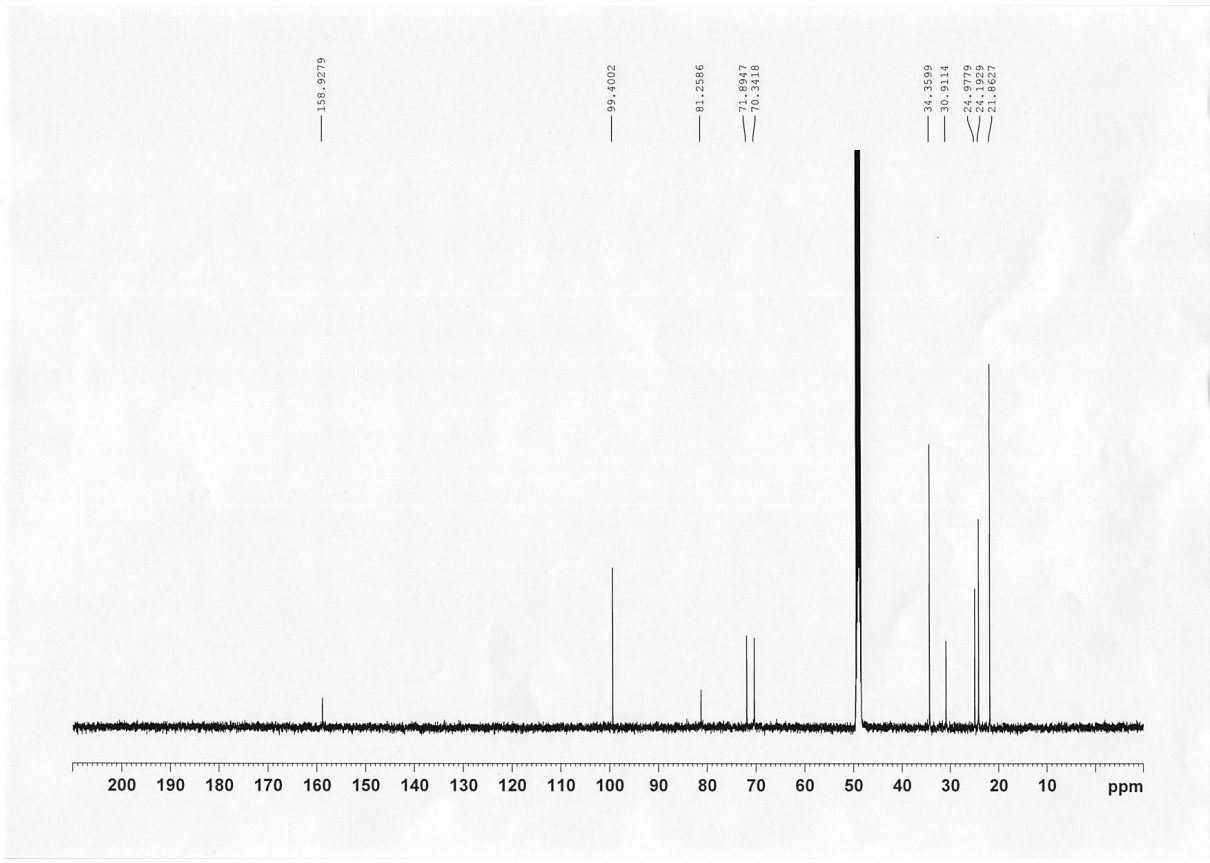
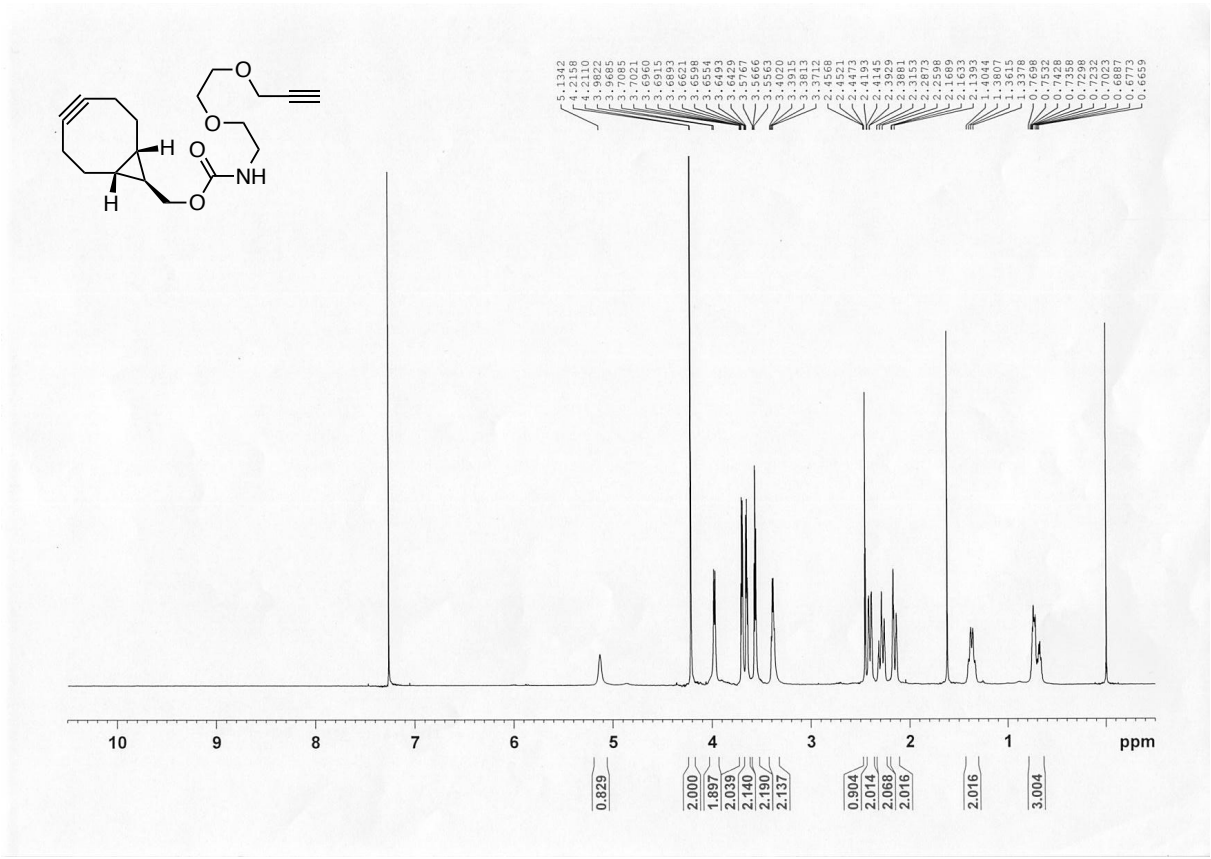
^{1}H and ^{13}C NMR Spectra of Compounds

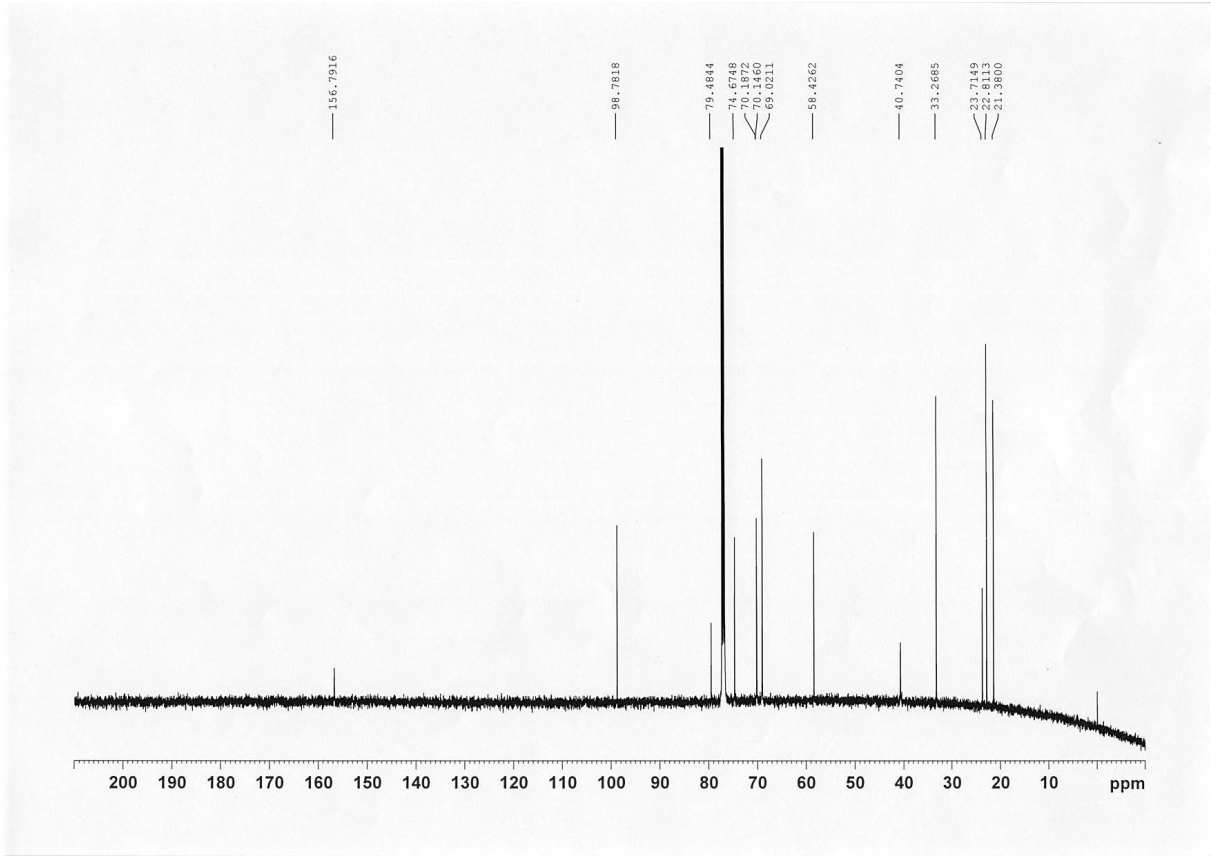
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **1b** (CD_3OD)



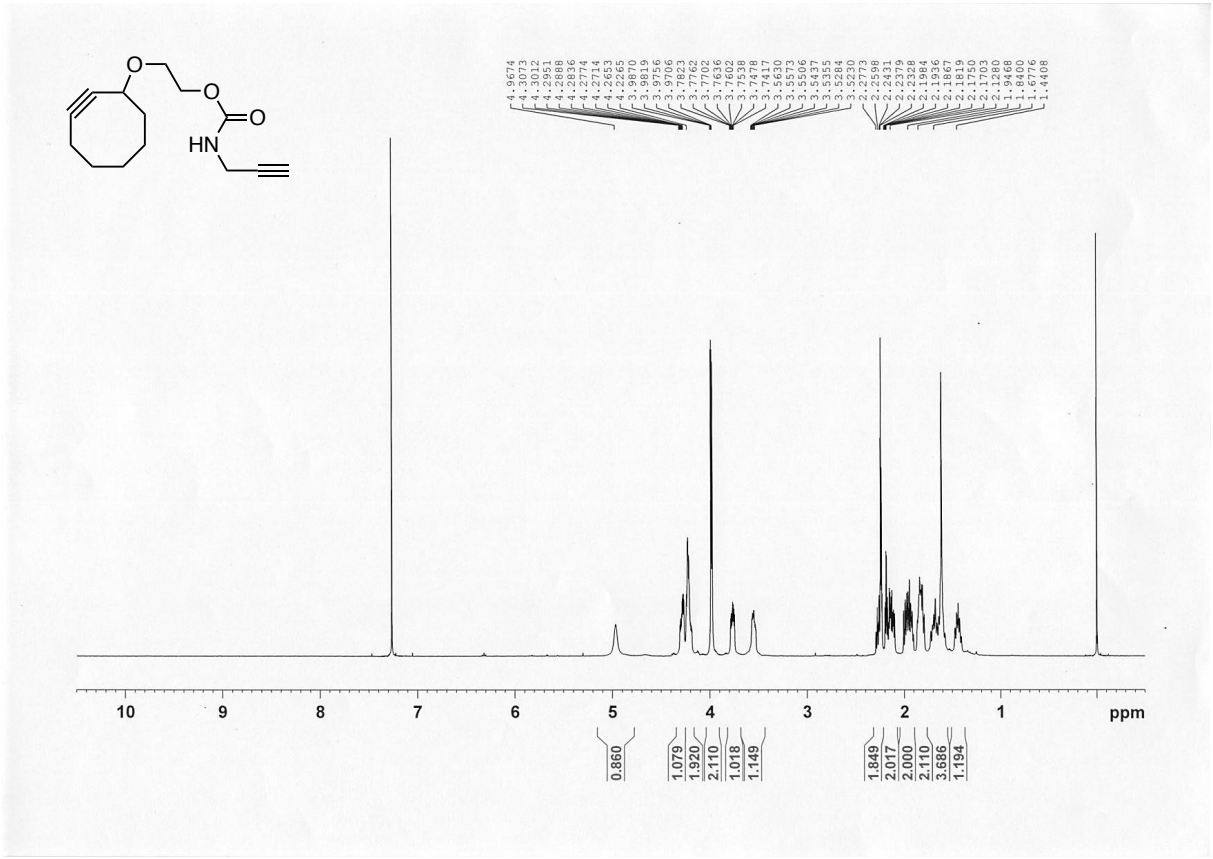


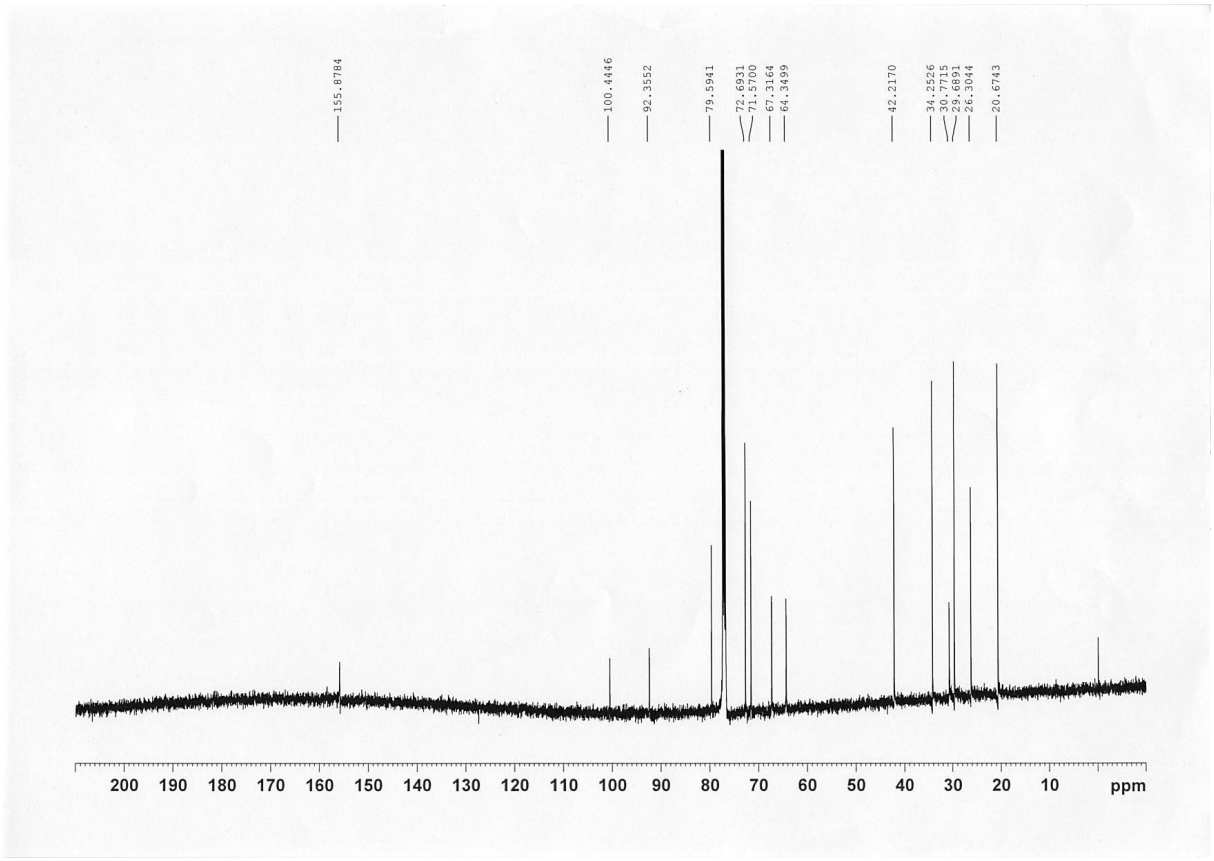
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **1c** ($CDCl_3$)



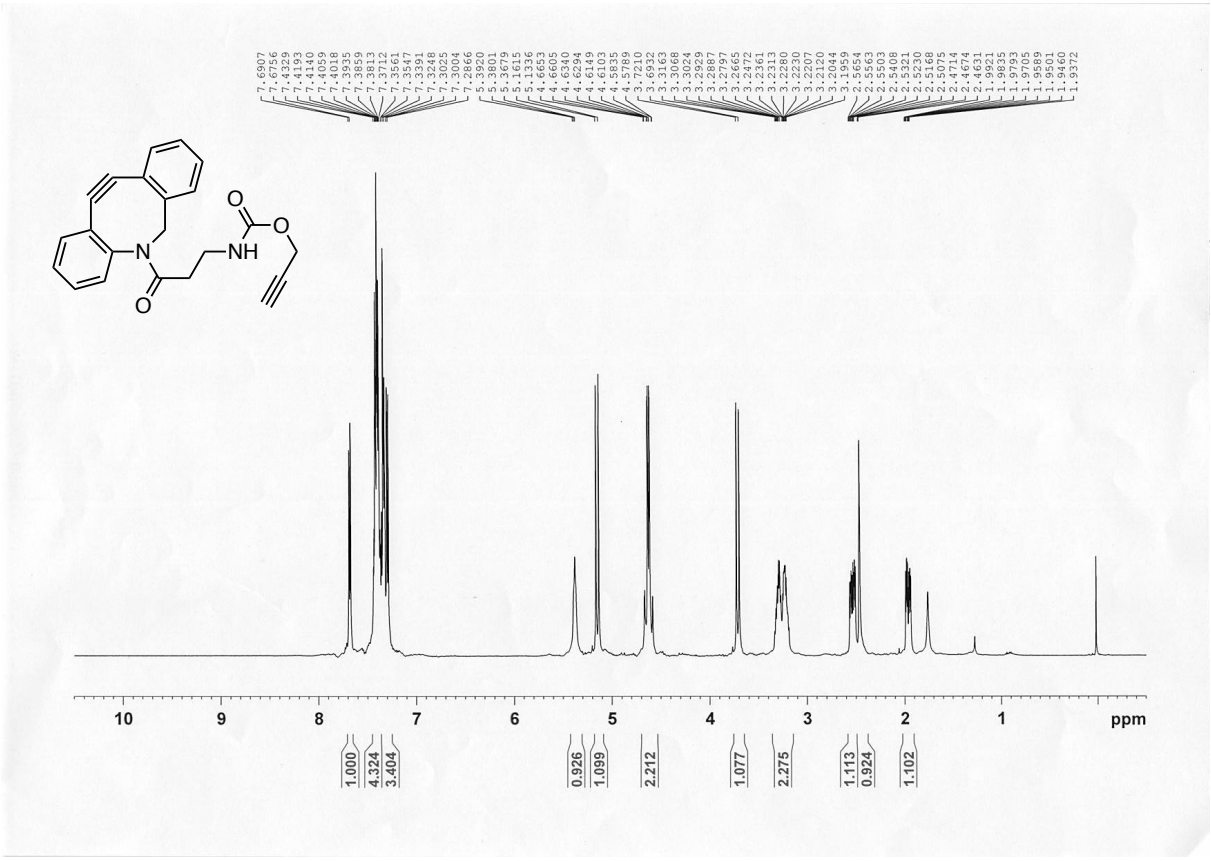


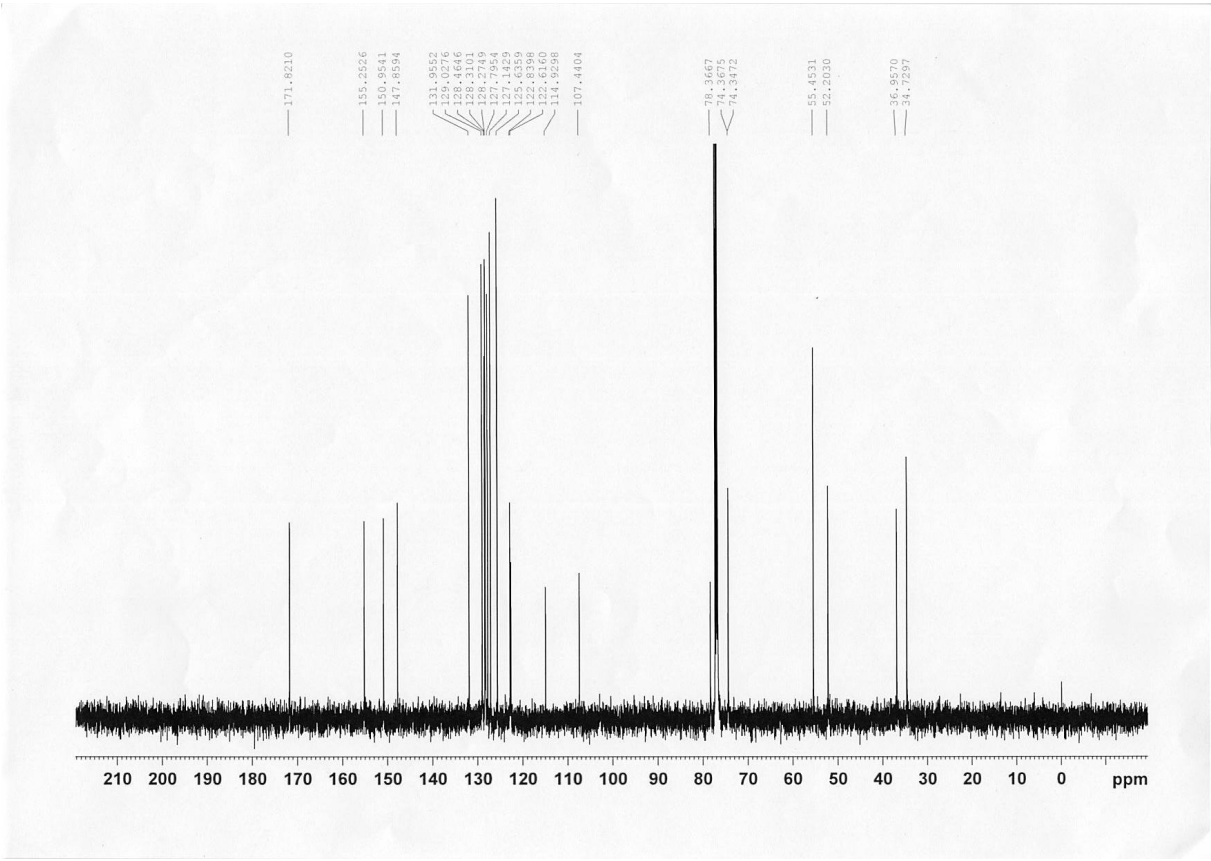
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **1d** ($CDCl_3$)



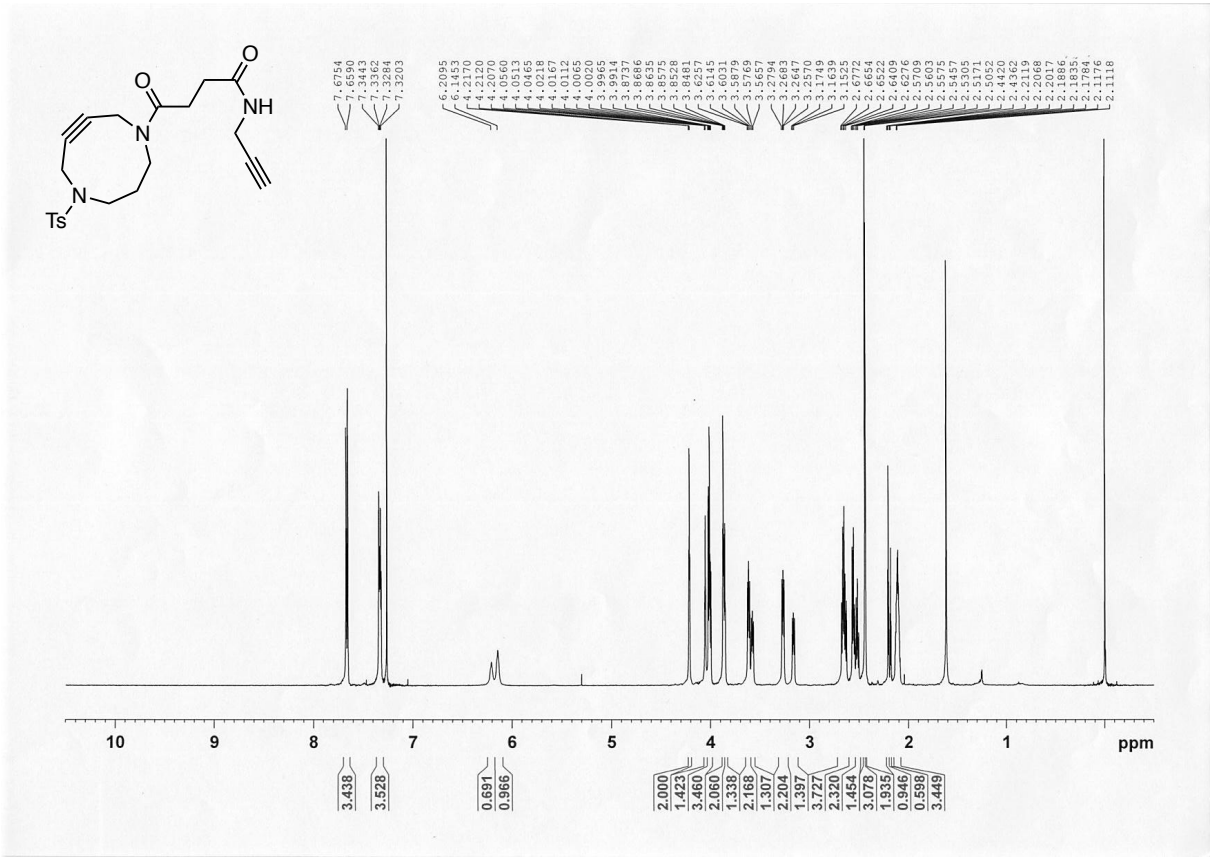


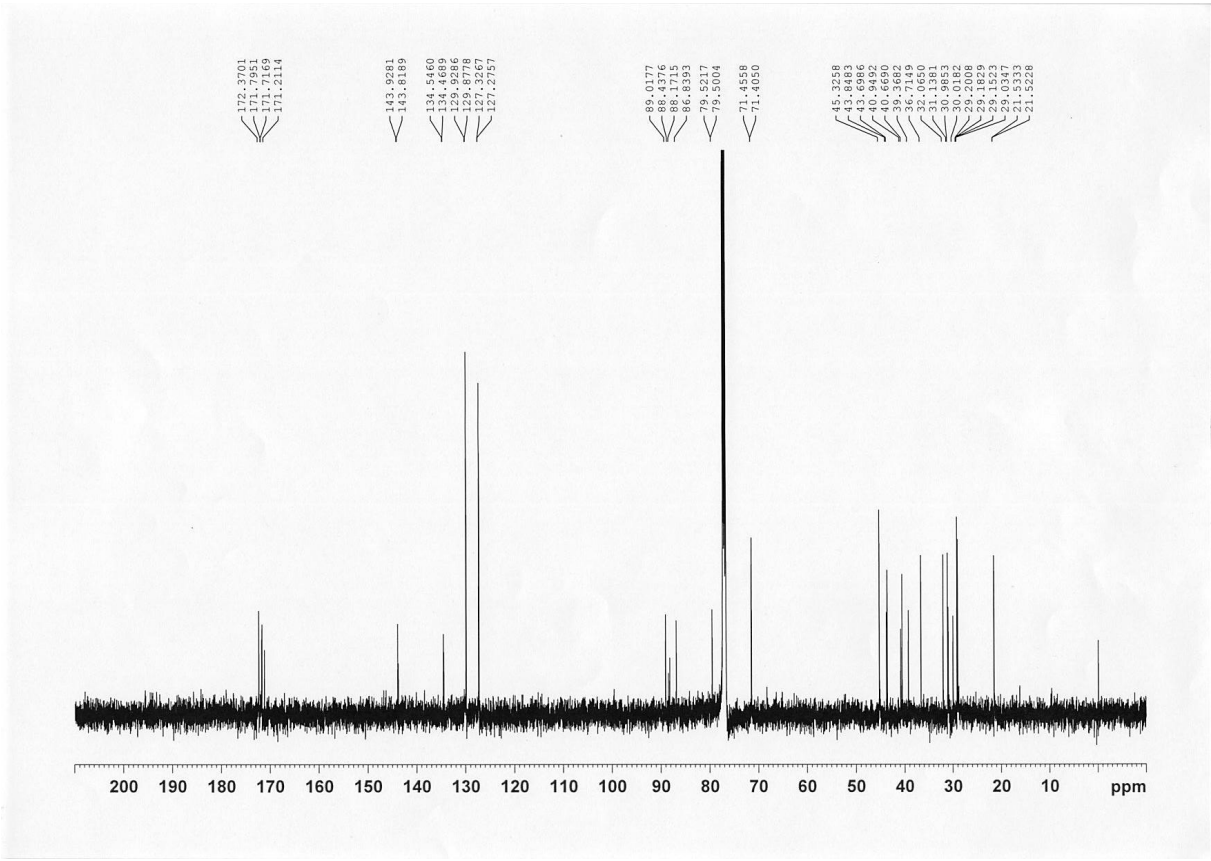
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **1e** ($CDCl_3$)



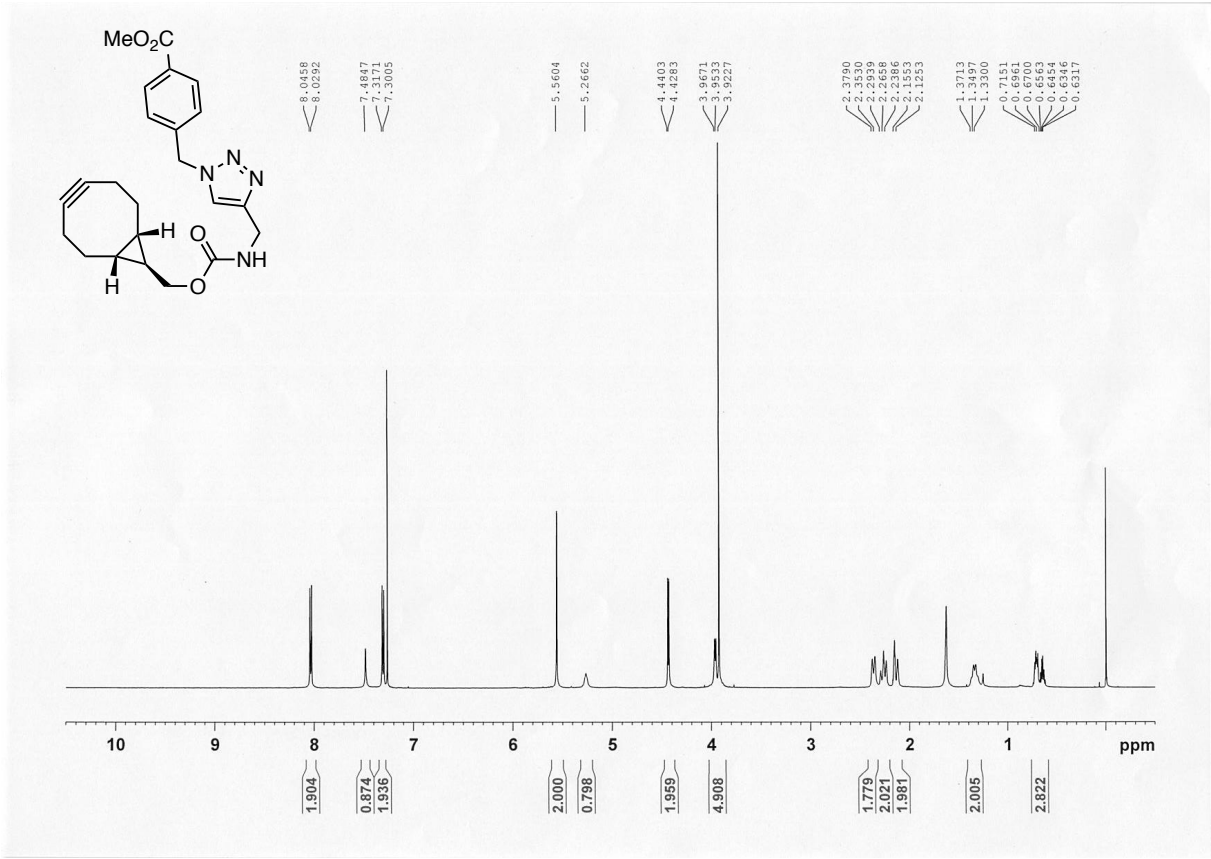


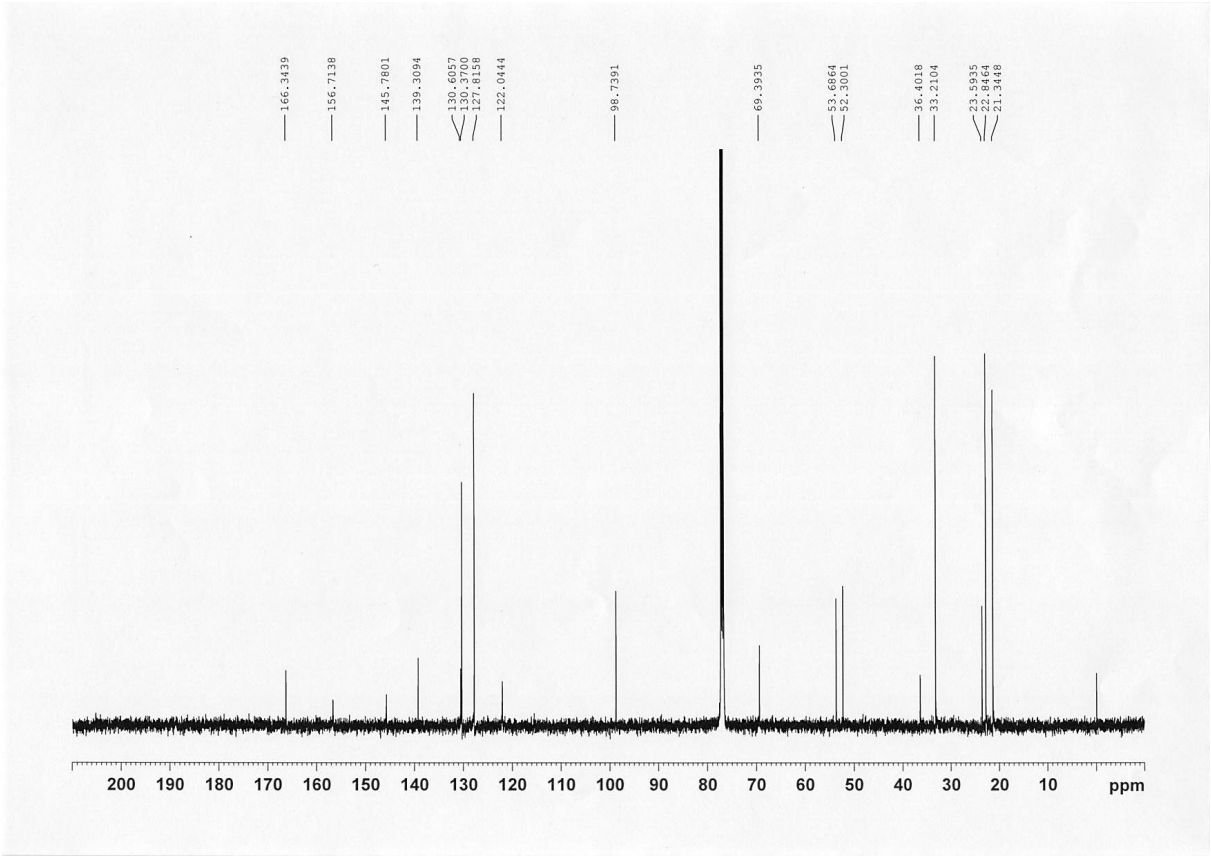
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **1f** ($CDCl_3$)



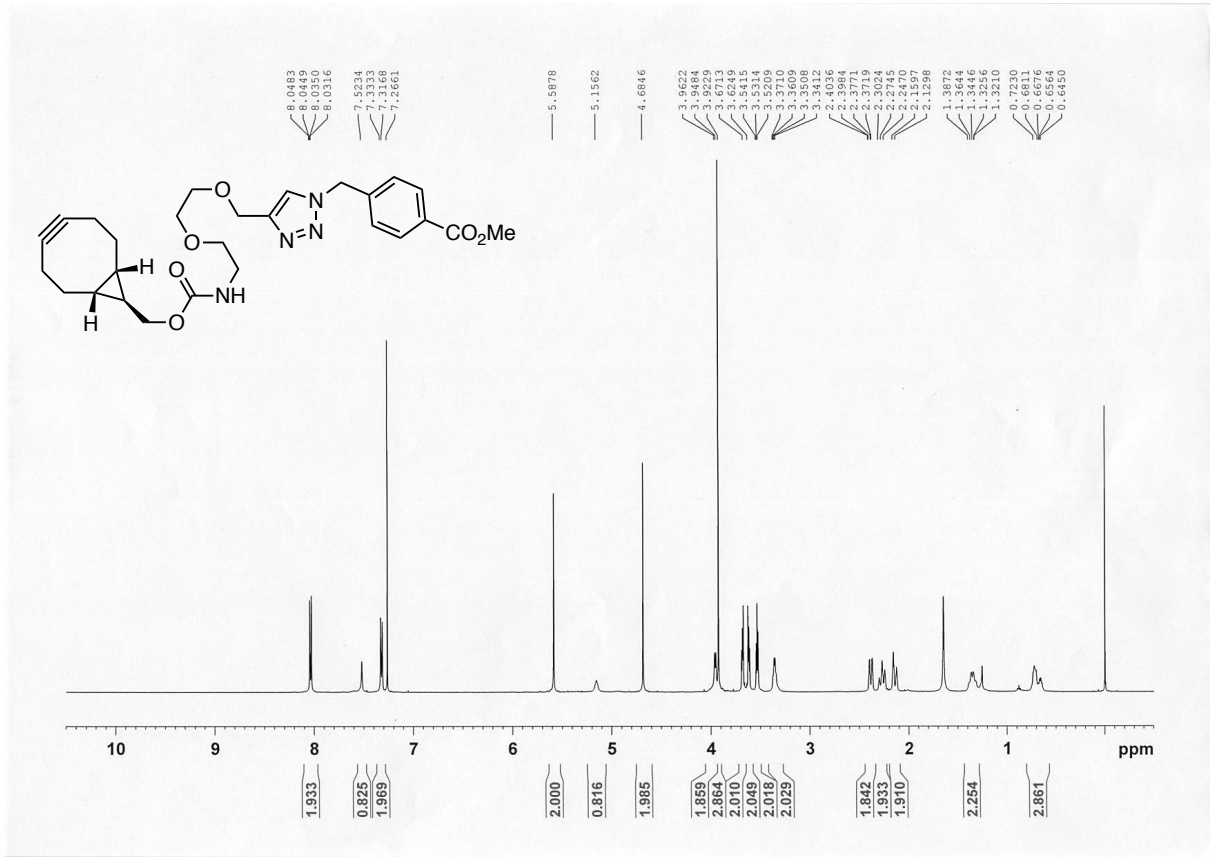


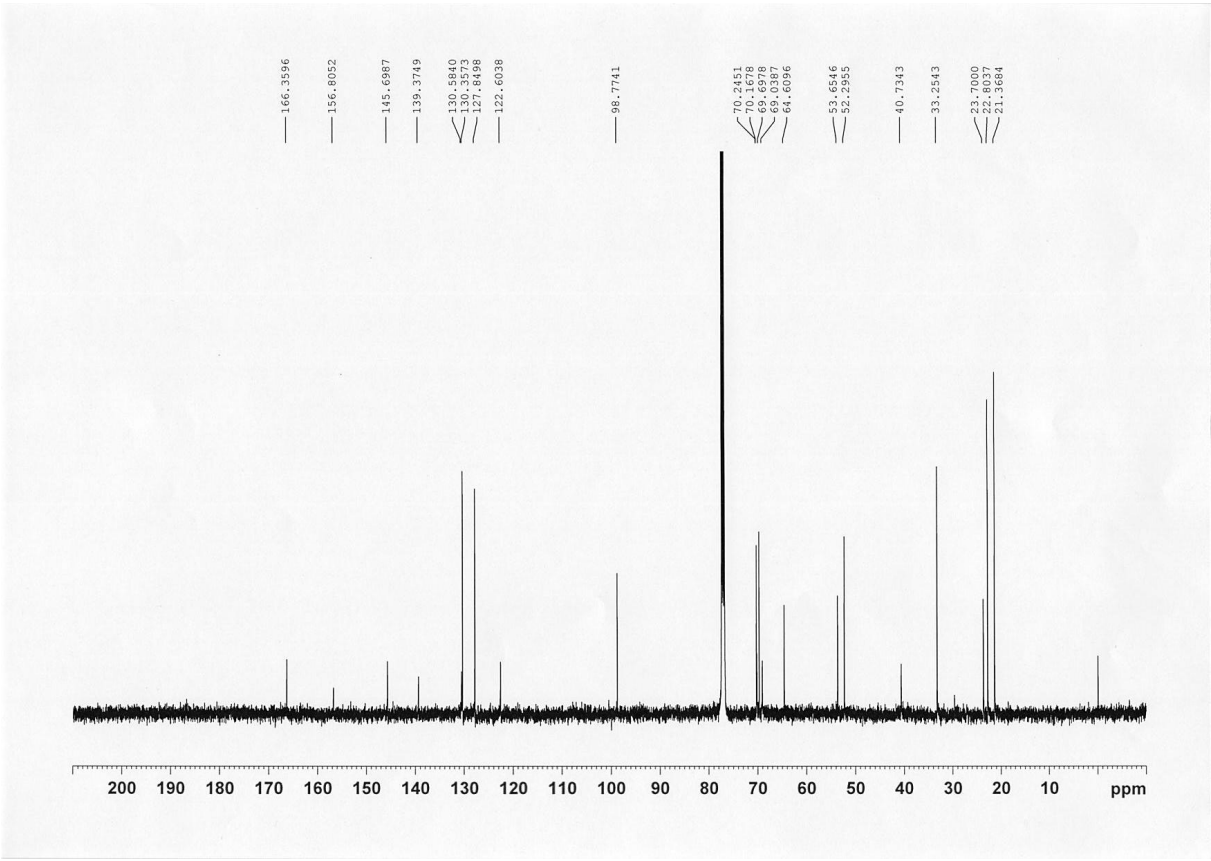
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4b** ($CDCl_3$)



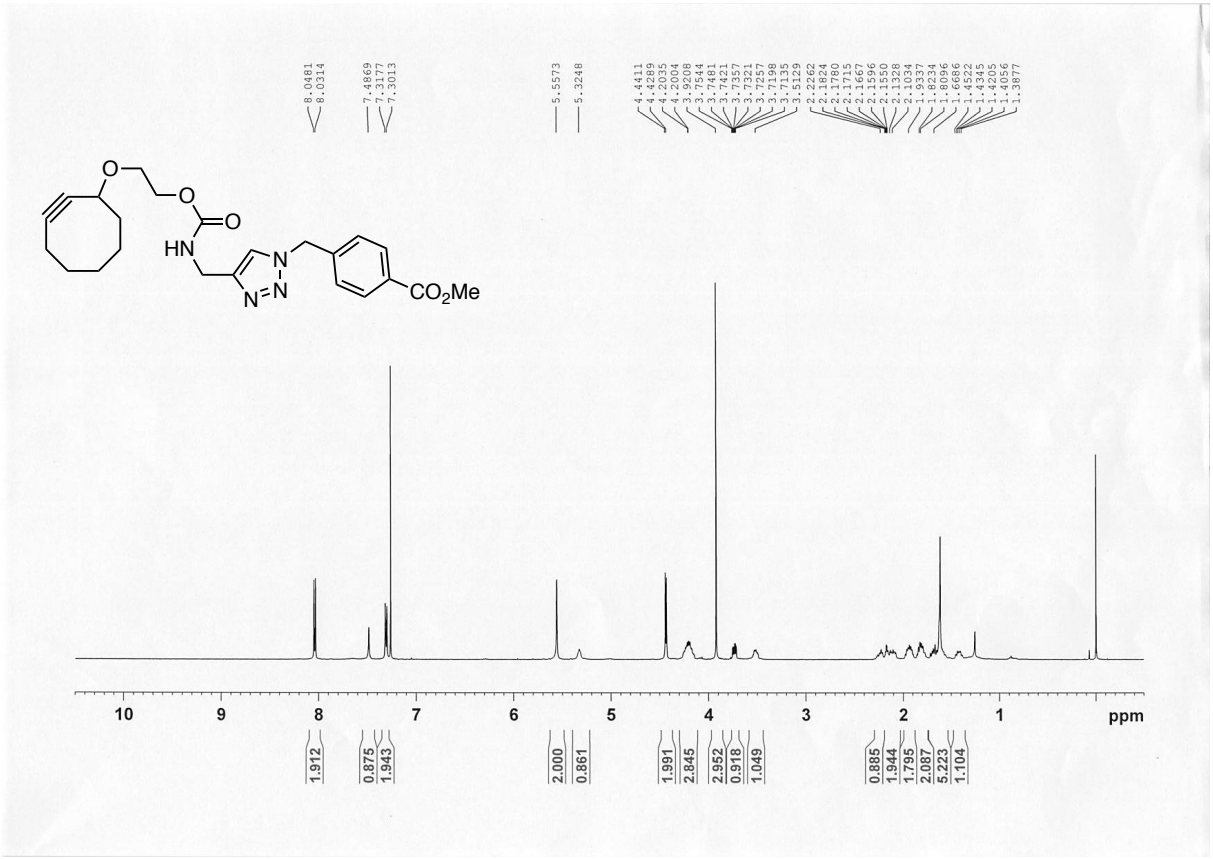


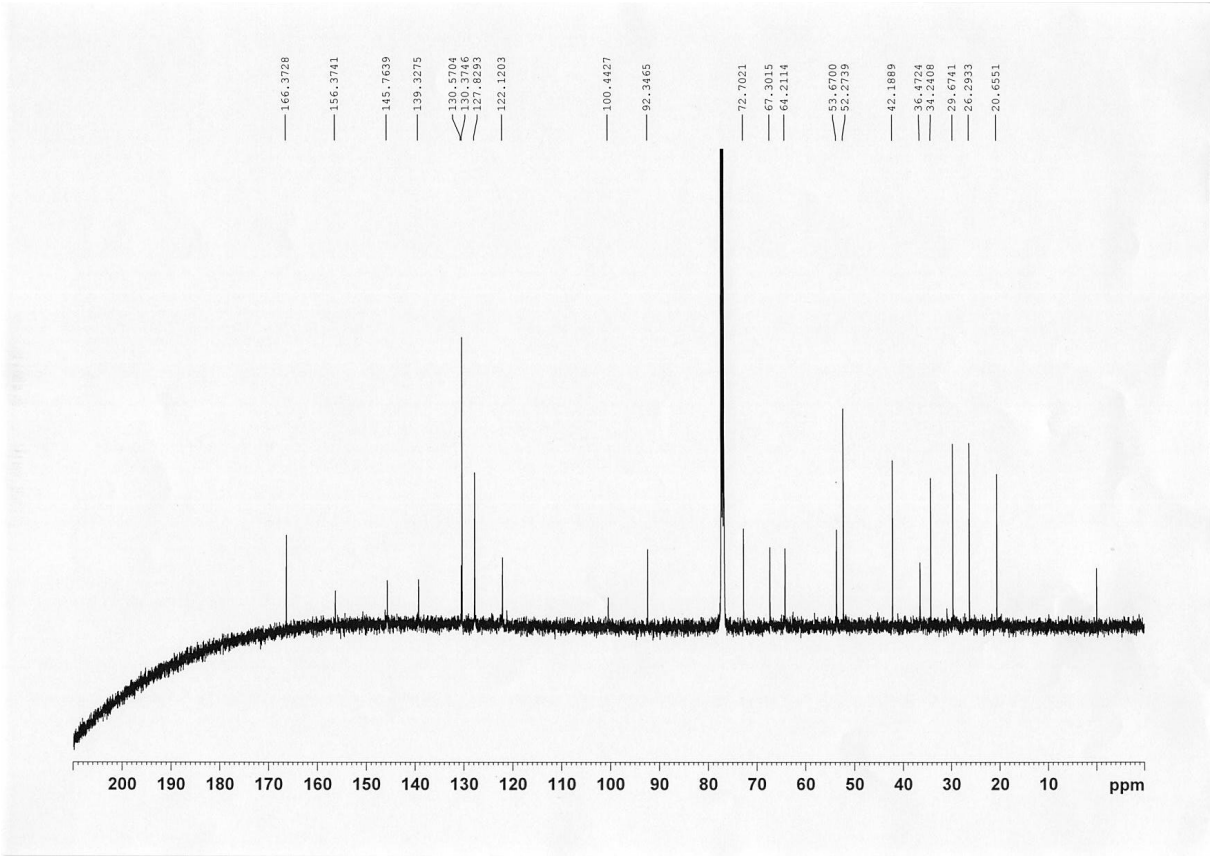
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4c** ($CDCl_3$)



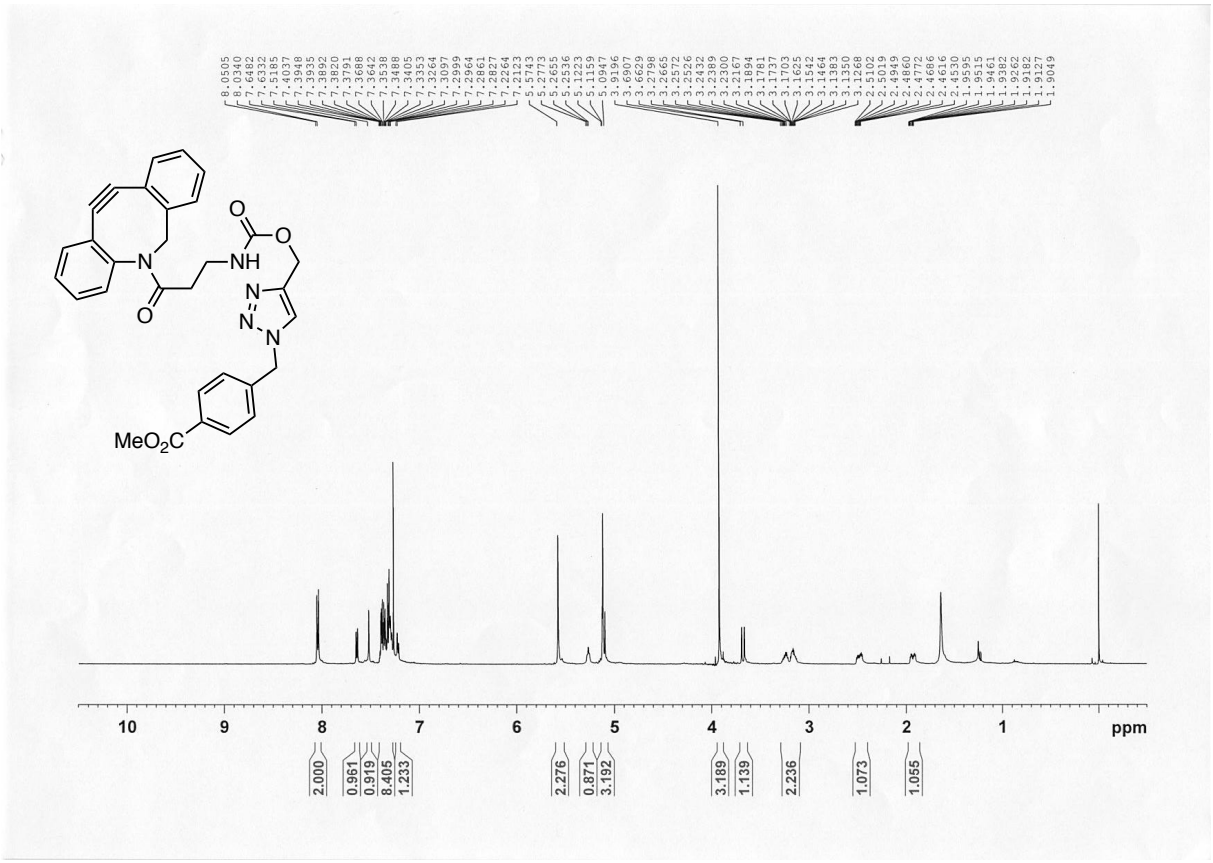


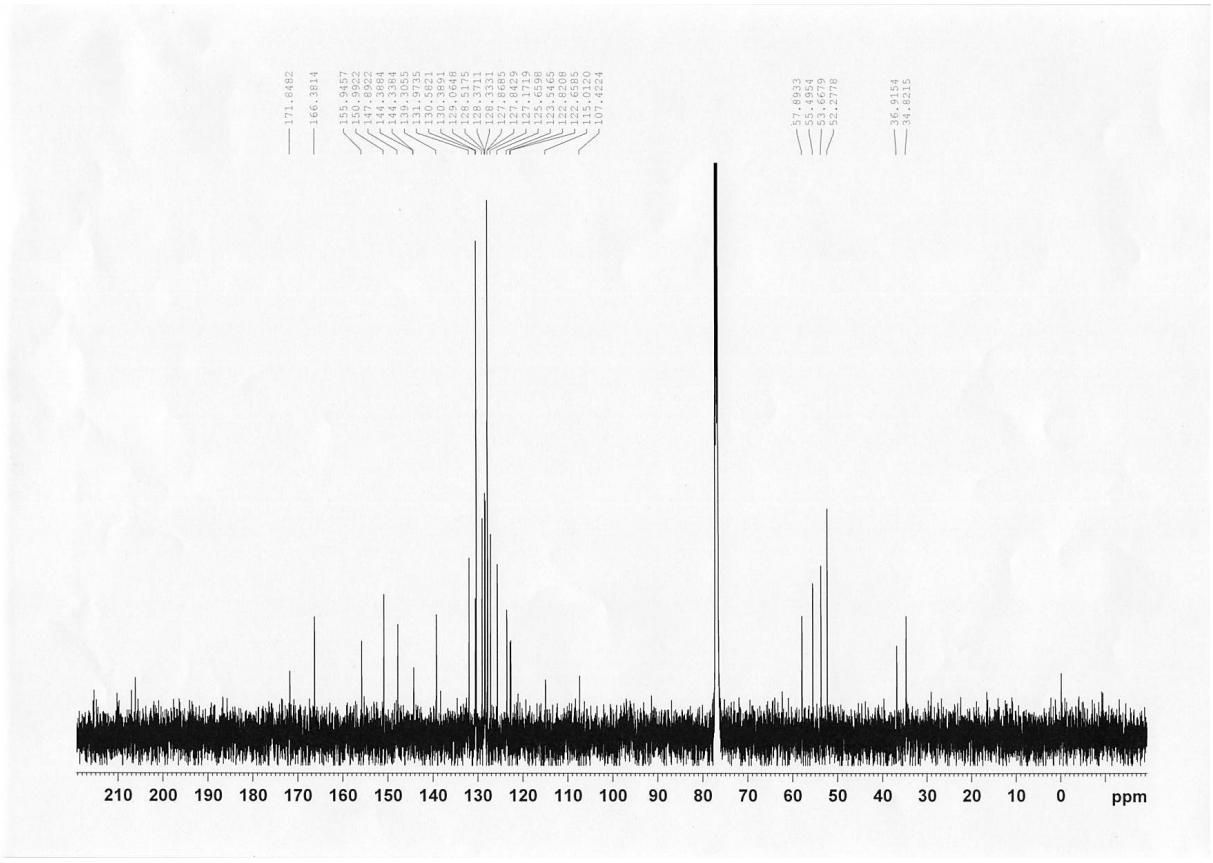
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4d** ($CDCl_3$)



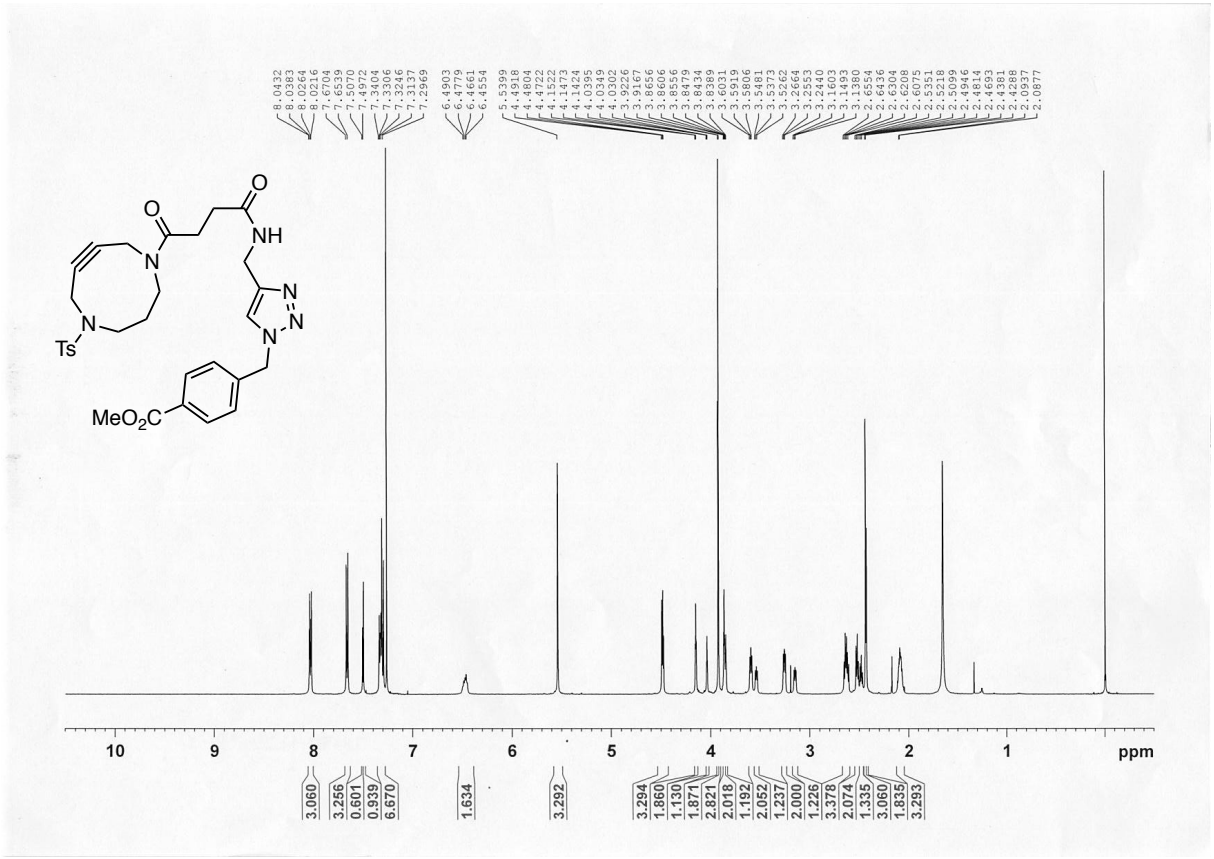


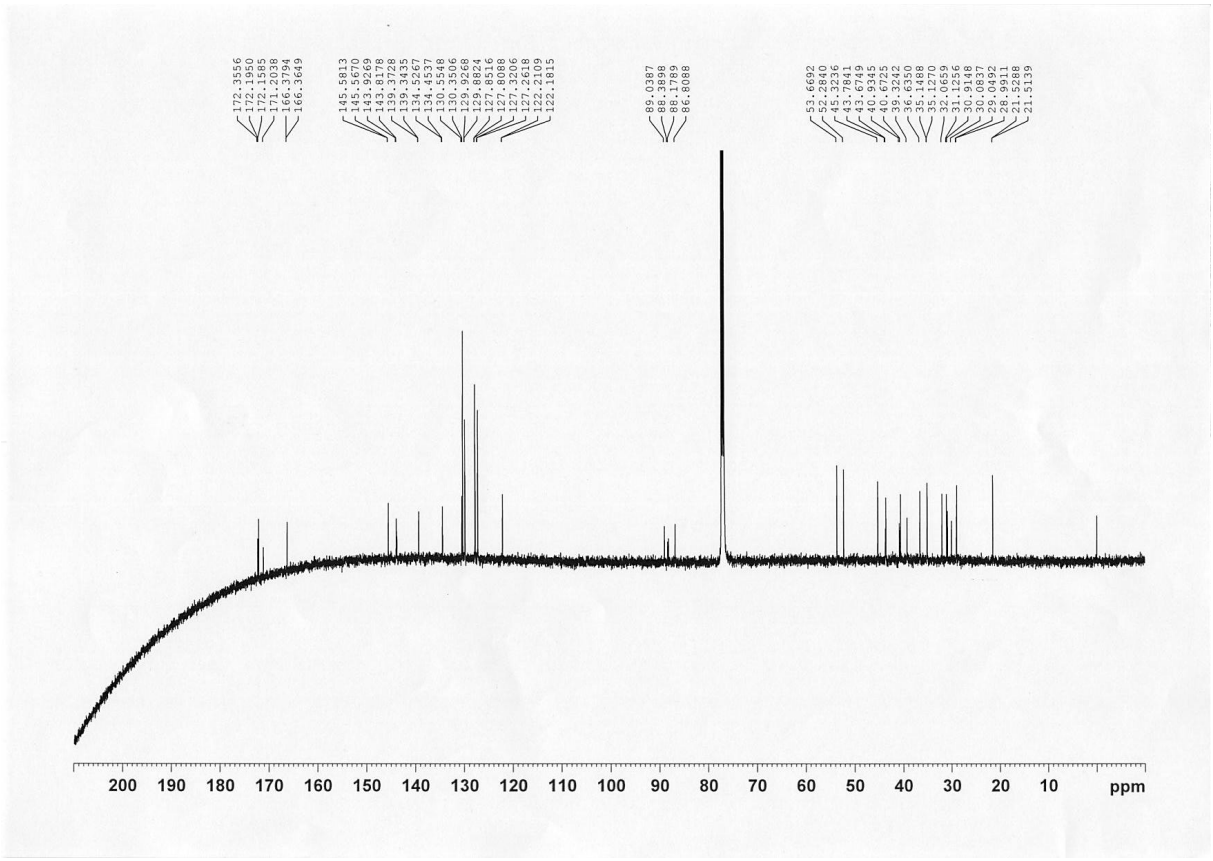
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4e** ($CDCl_3$)



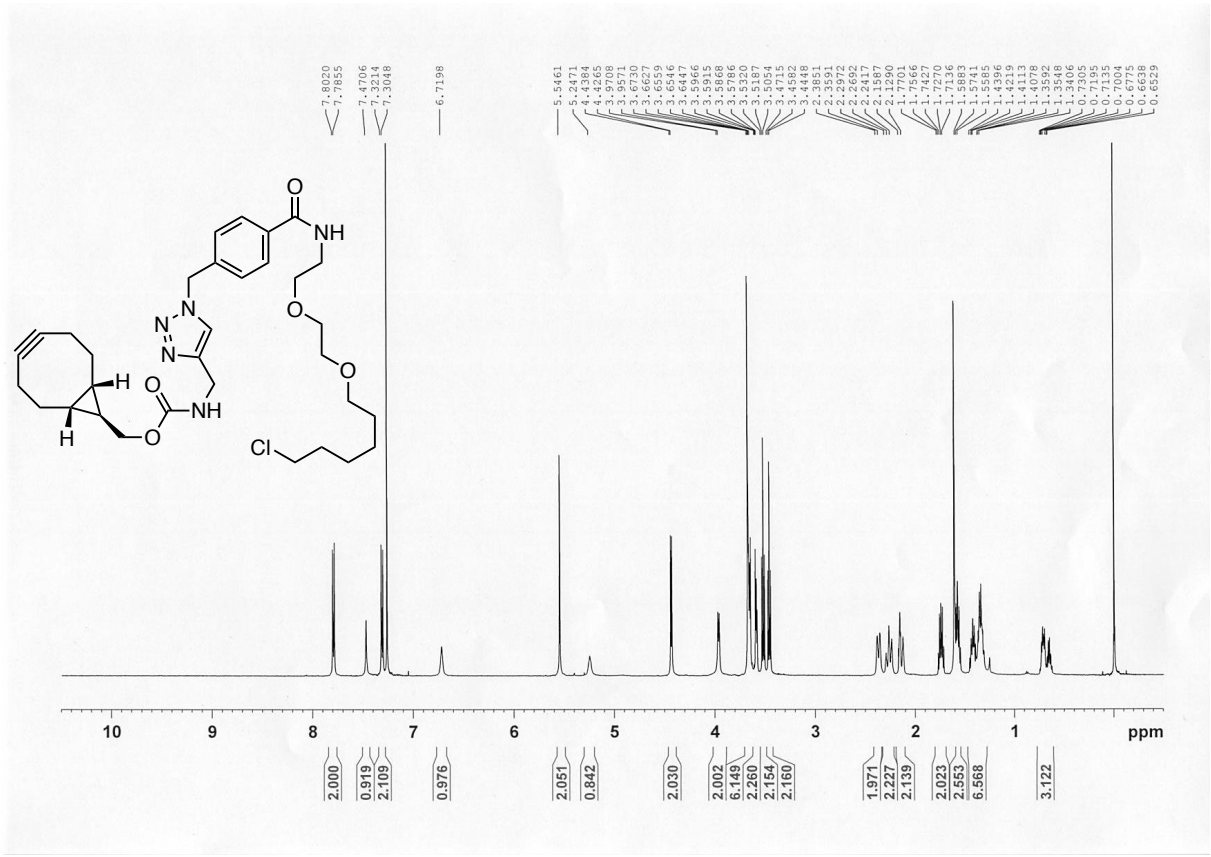


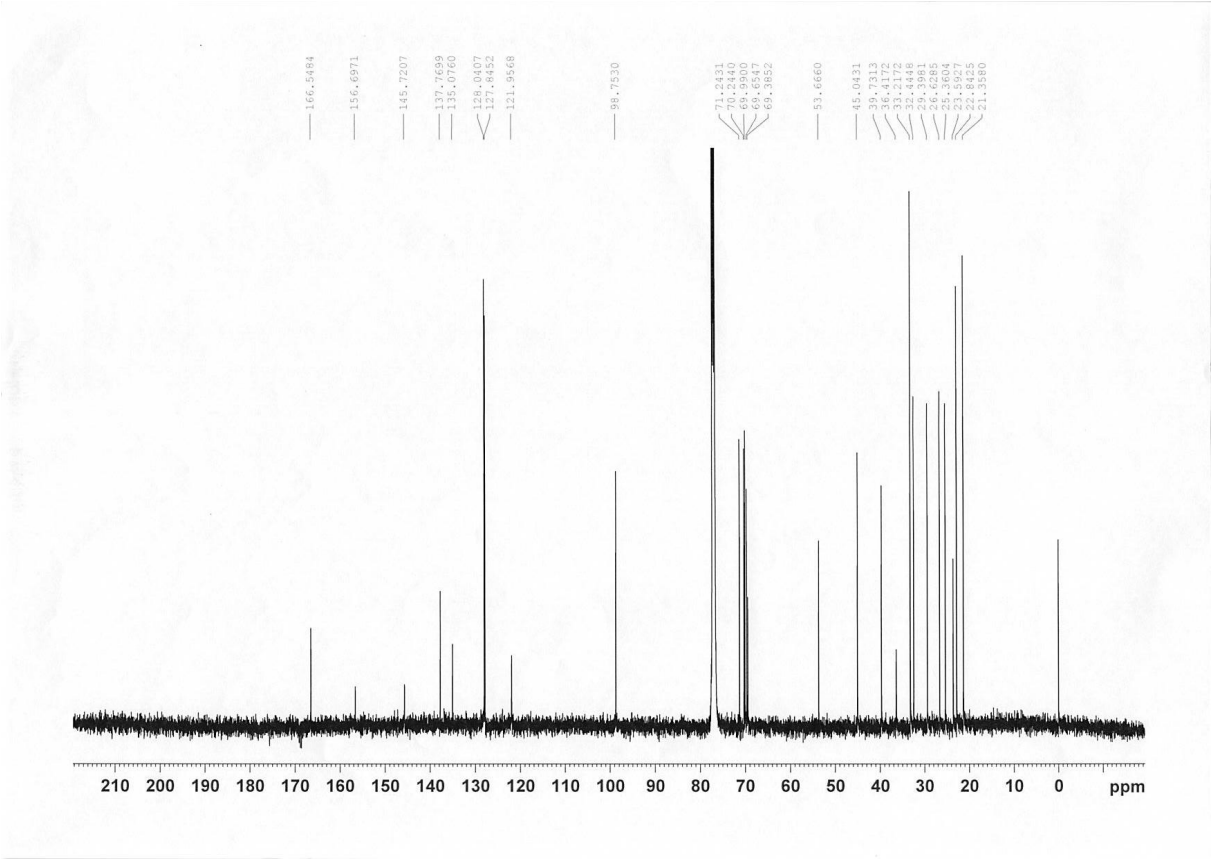
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4f** ($CDCl_3$)



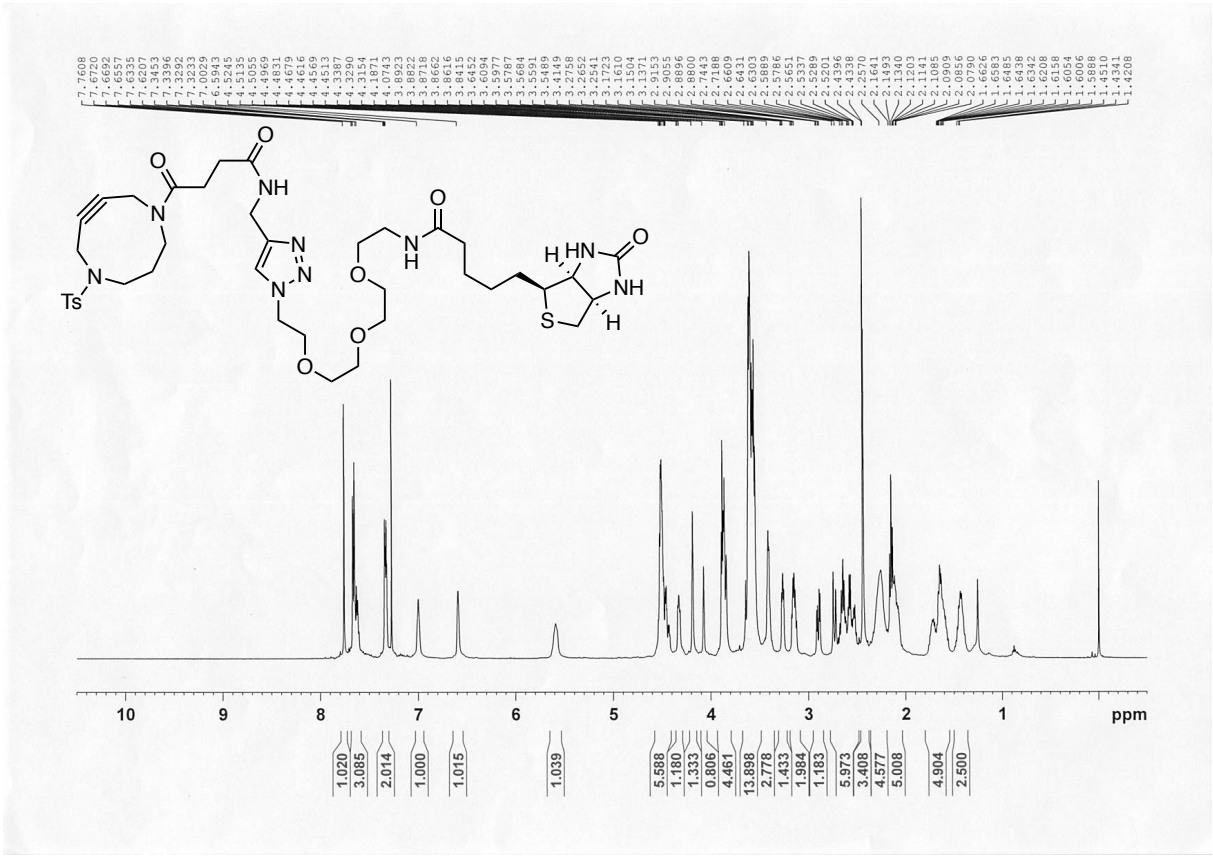


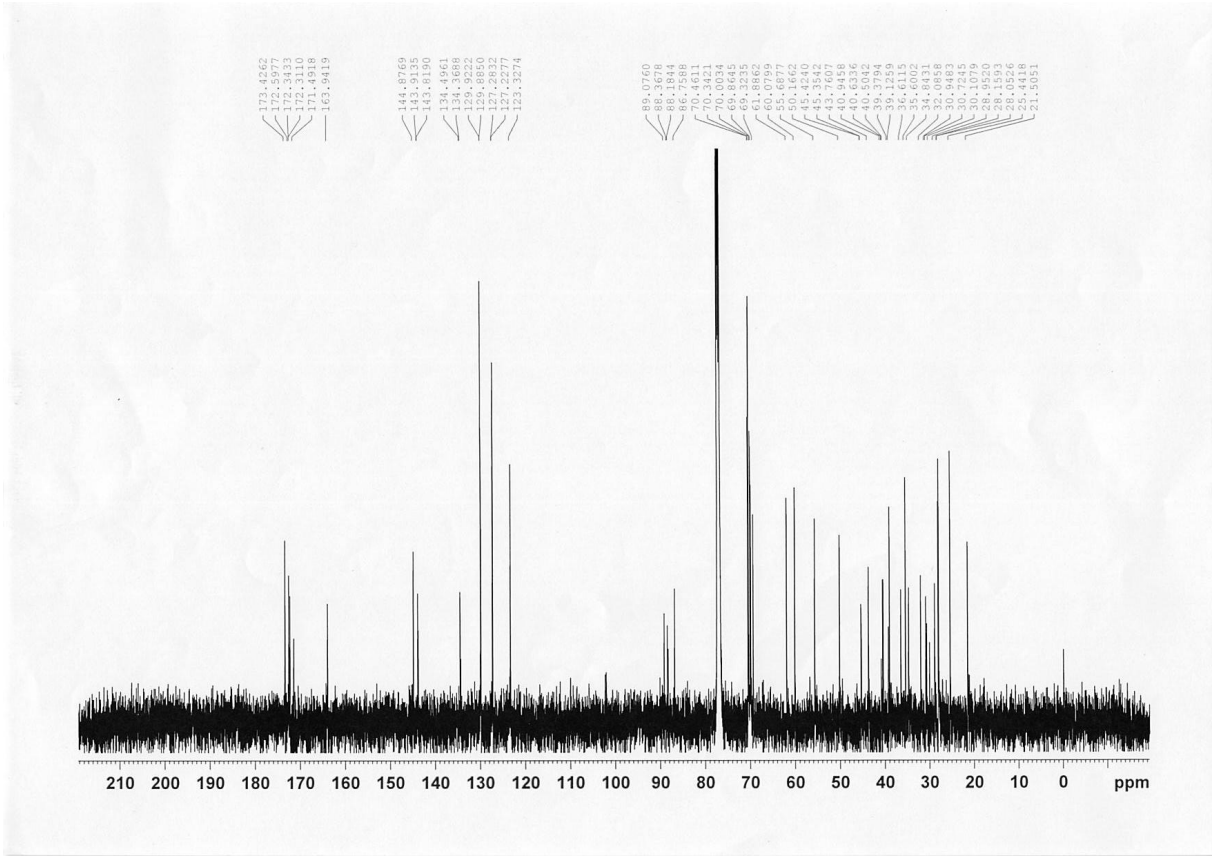
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4g** ($CDCl_3$)



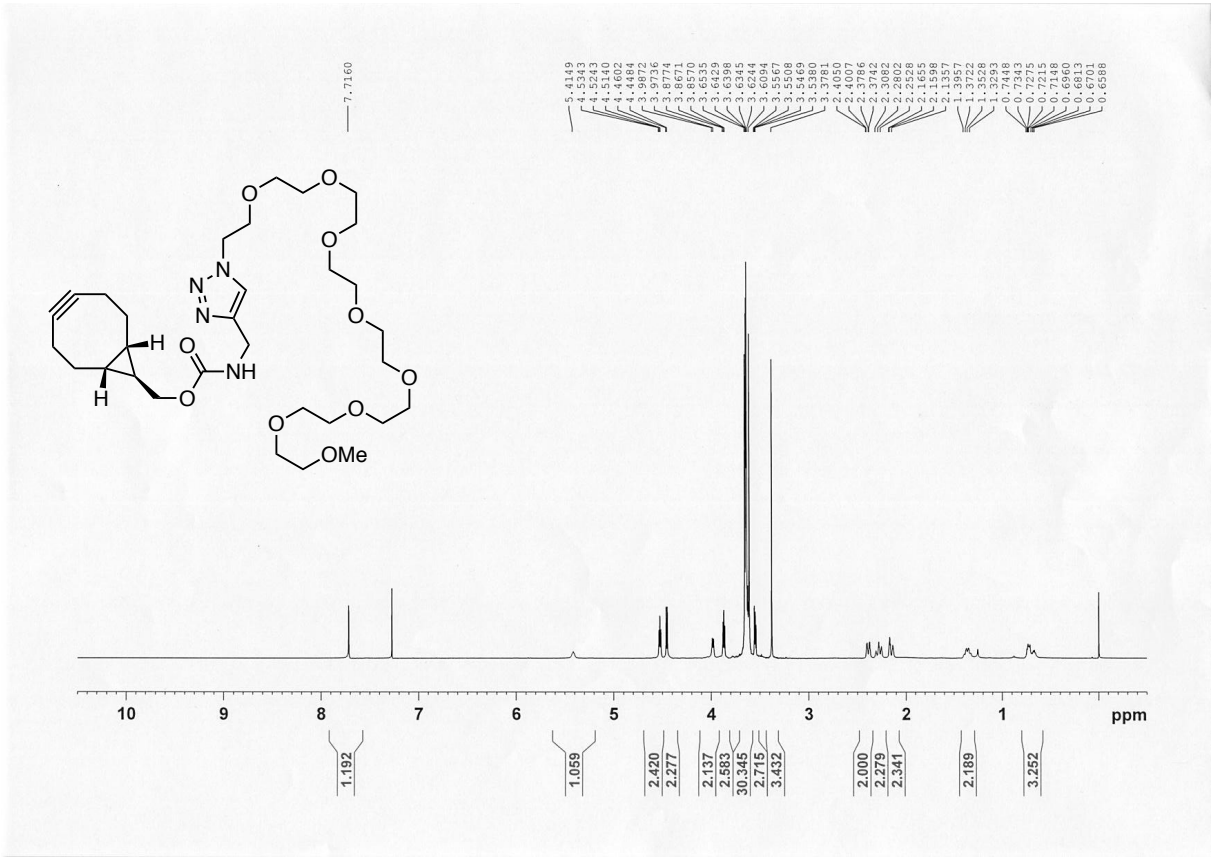


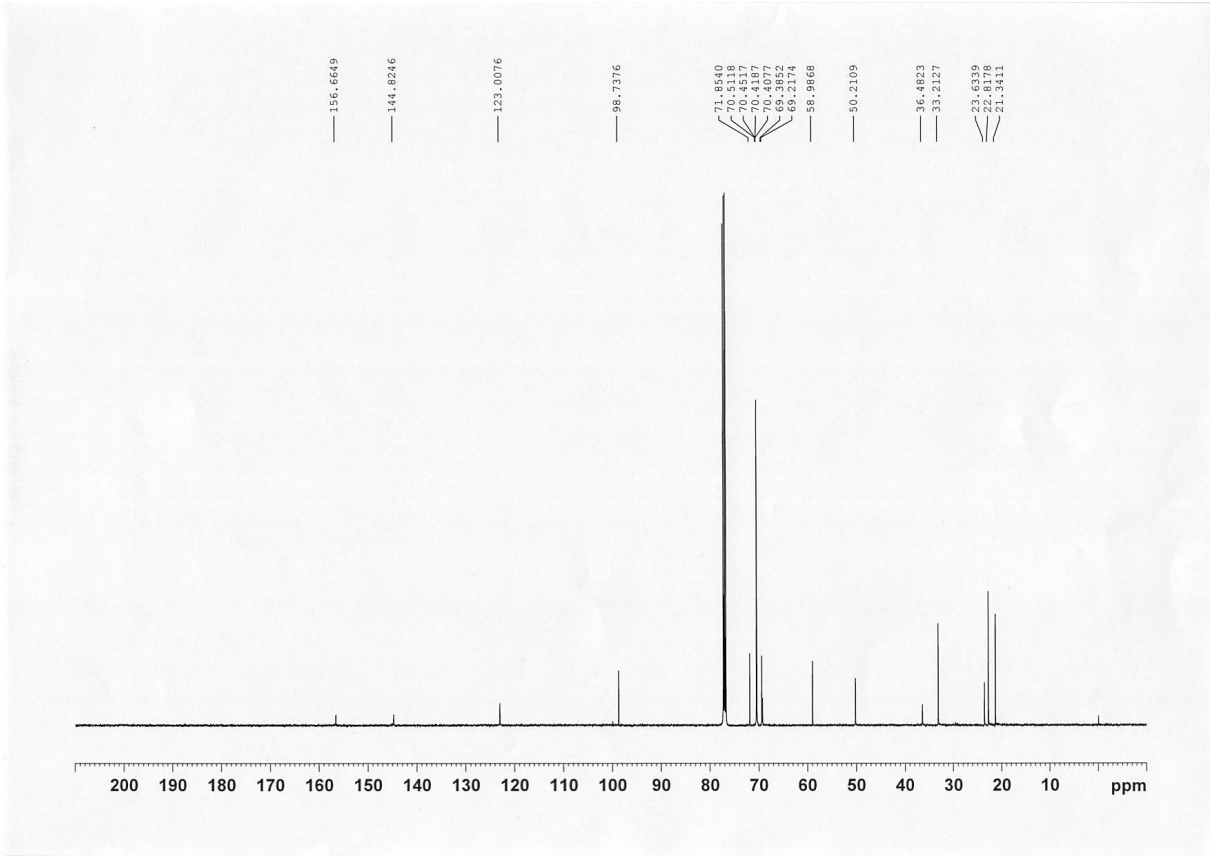
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4h** ($CDCl_3$)



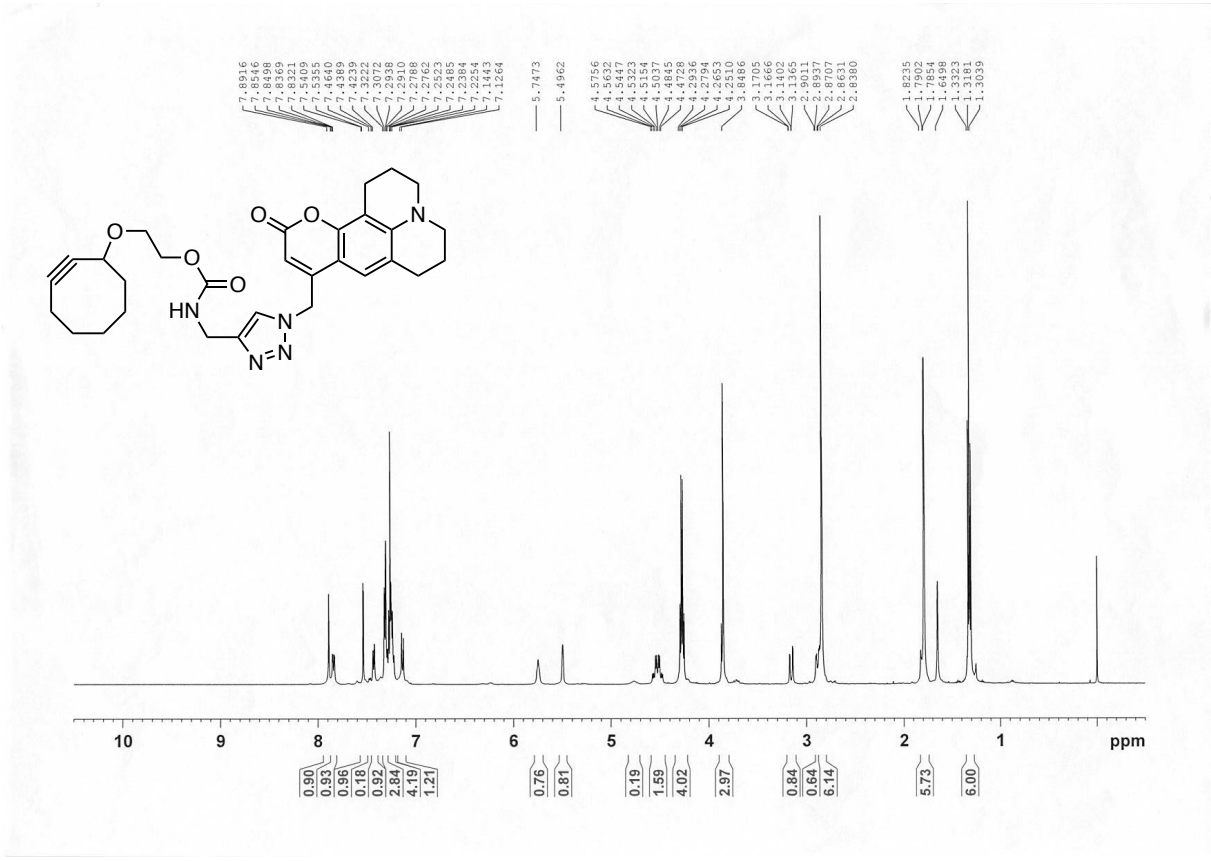


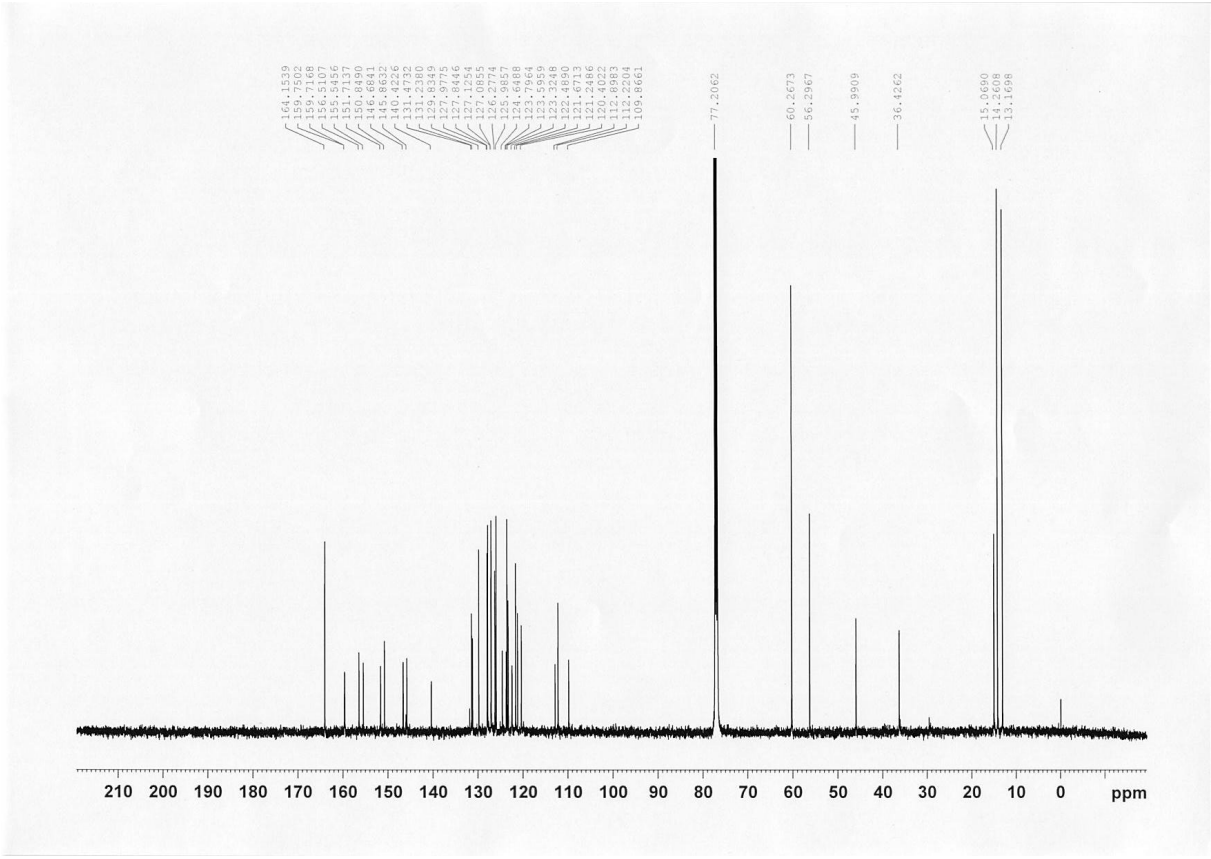
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4i** ($CDCl_3$)



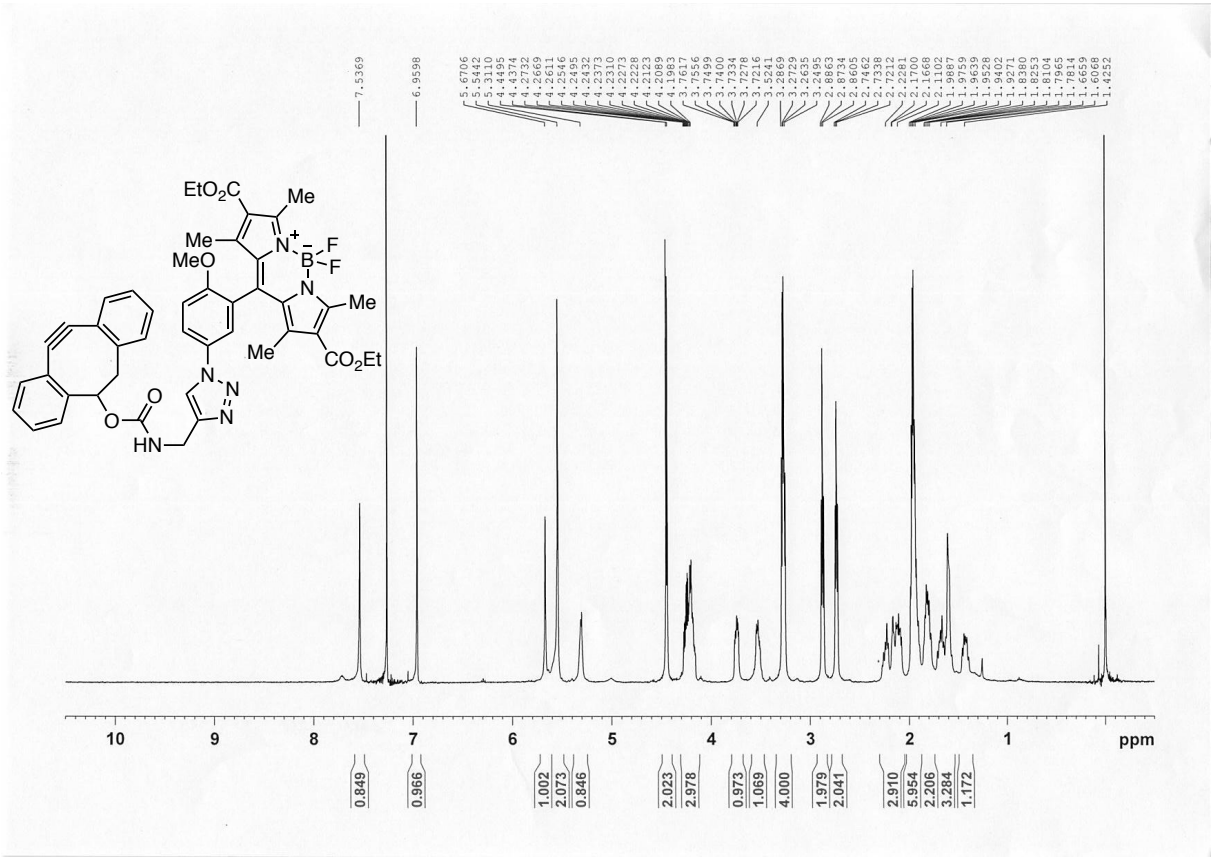


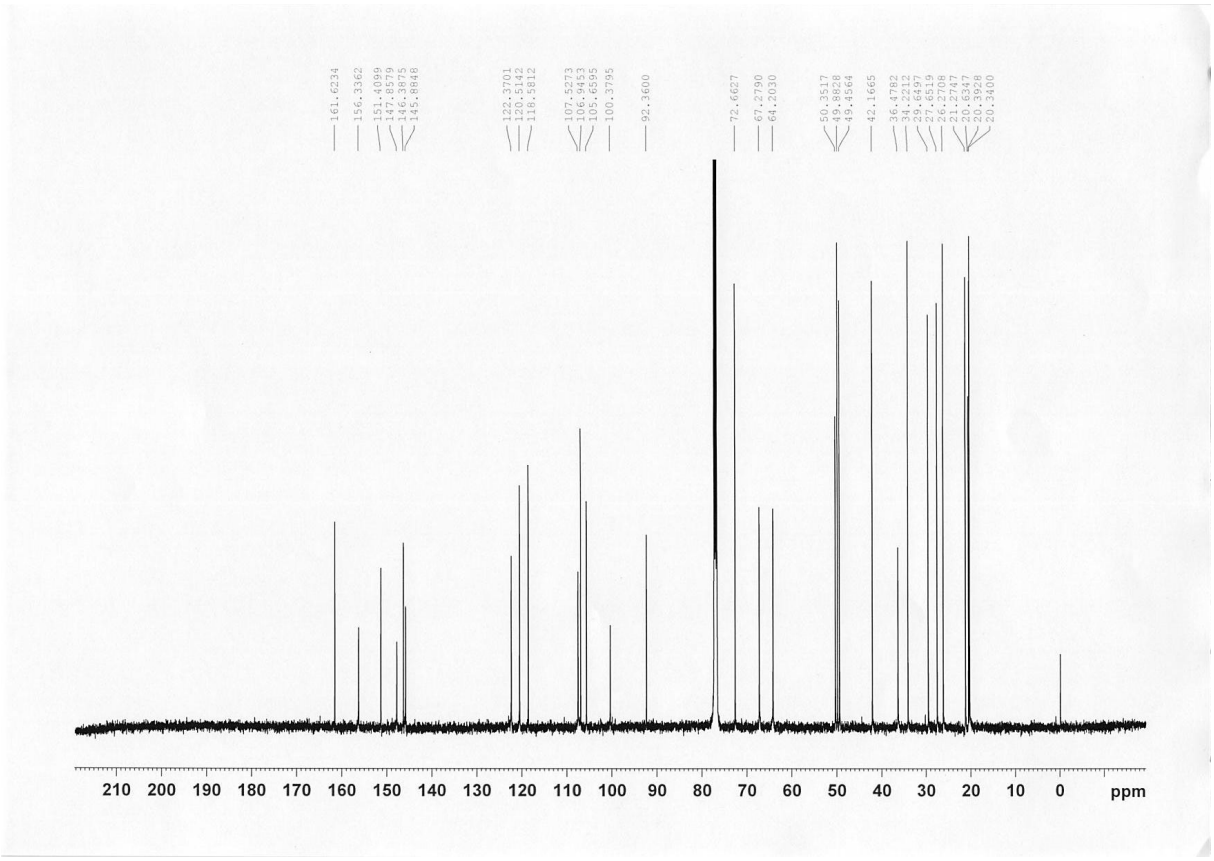
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4j** ($CDCl_3$)



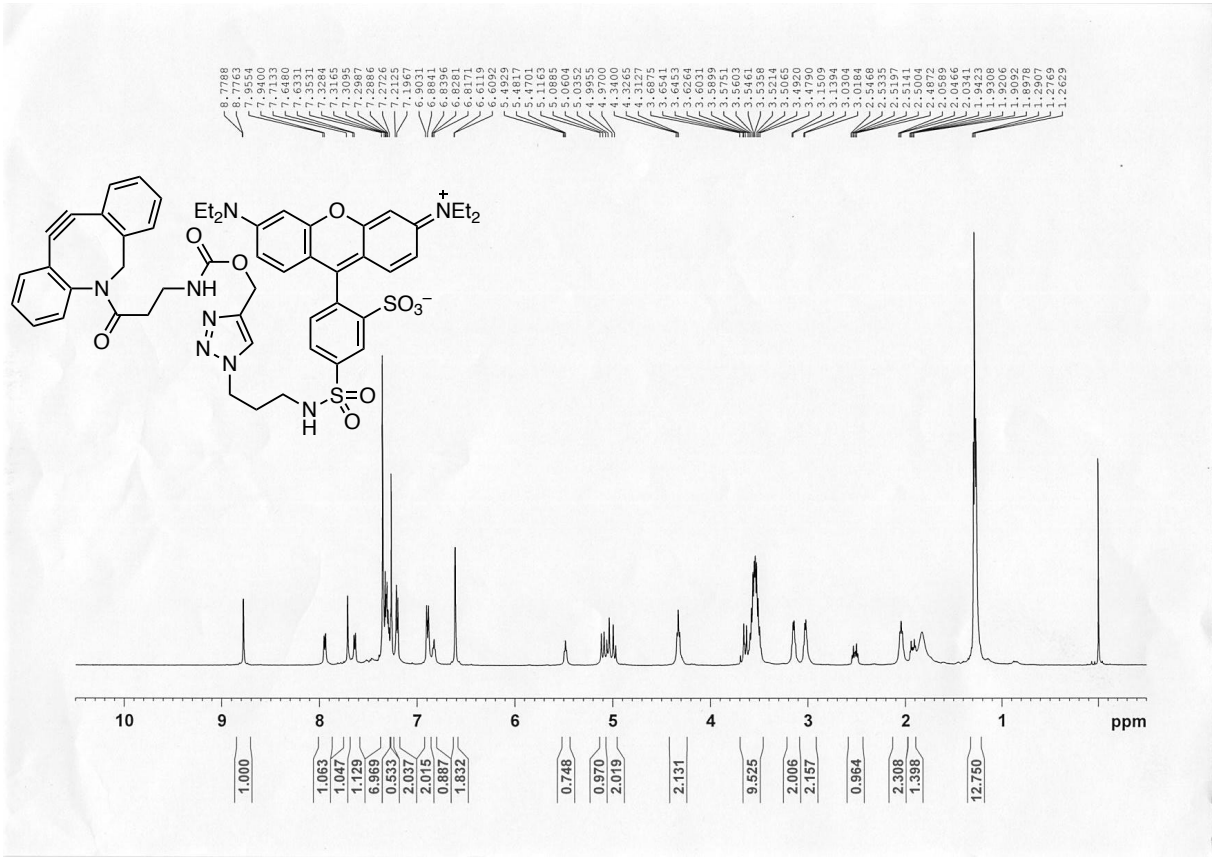


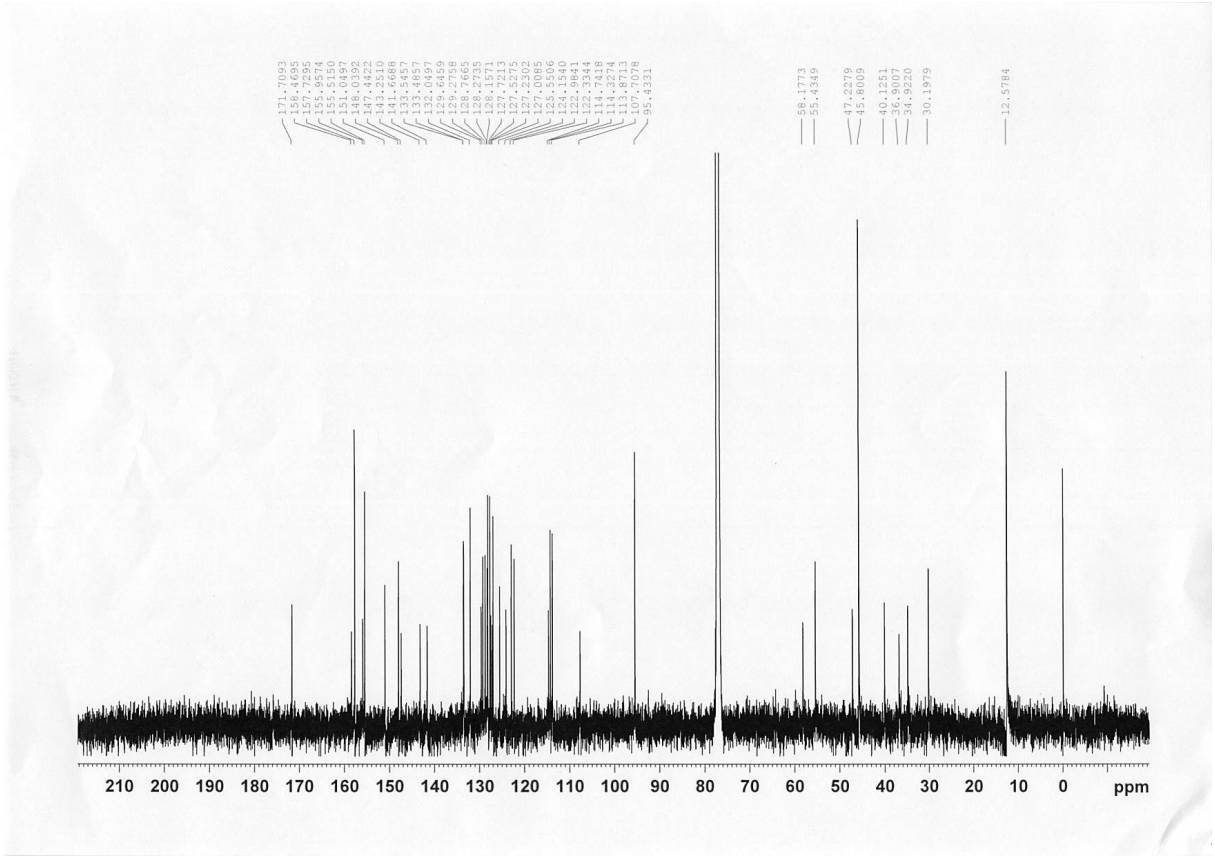
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4k** ($CDCl_3$)



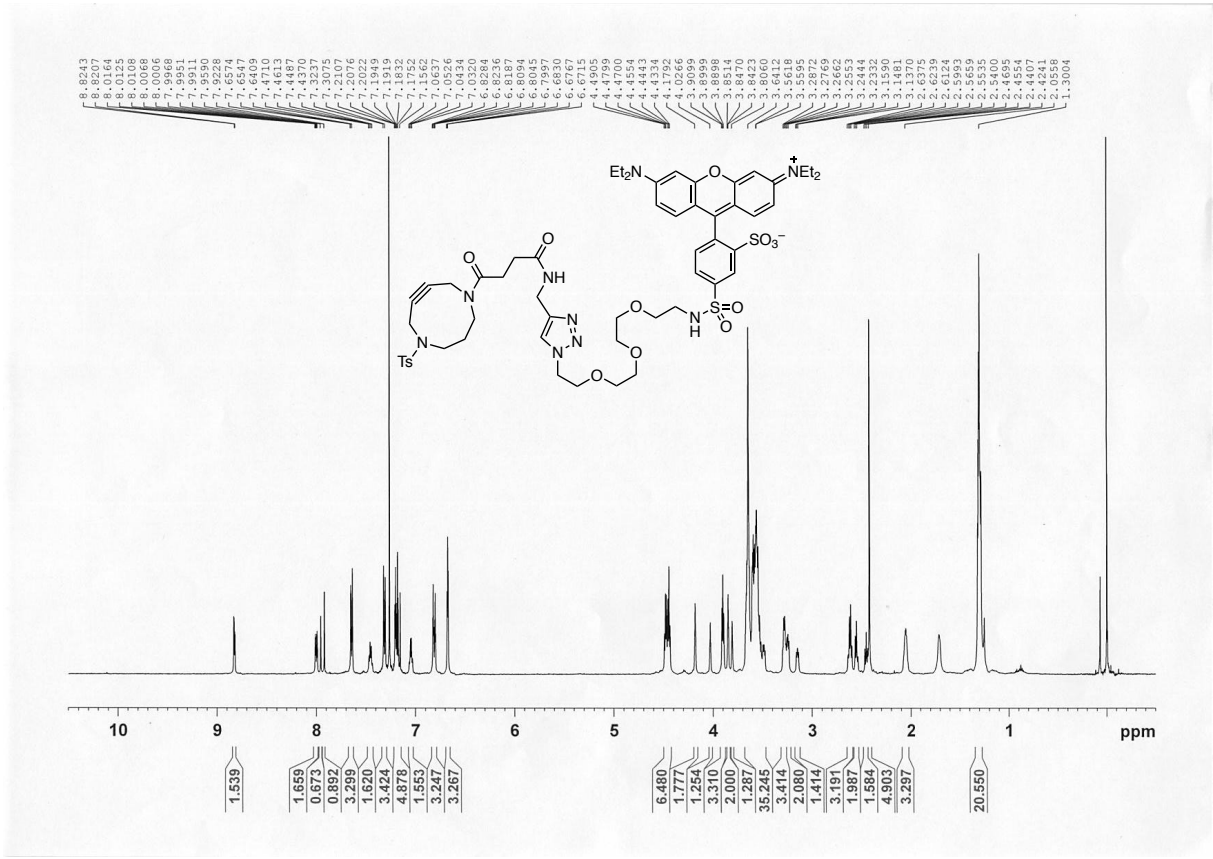


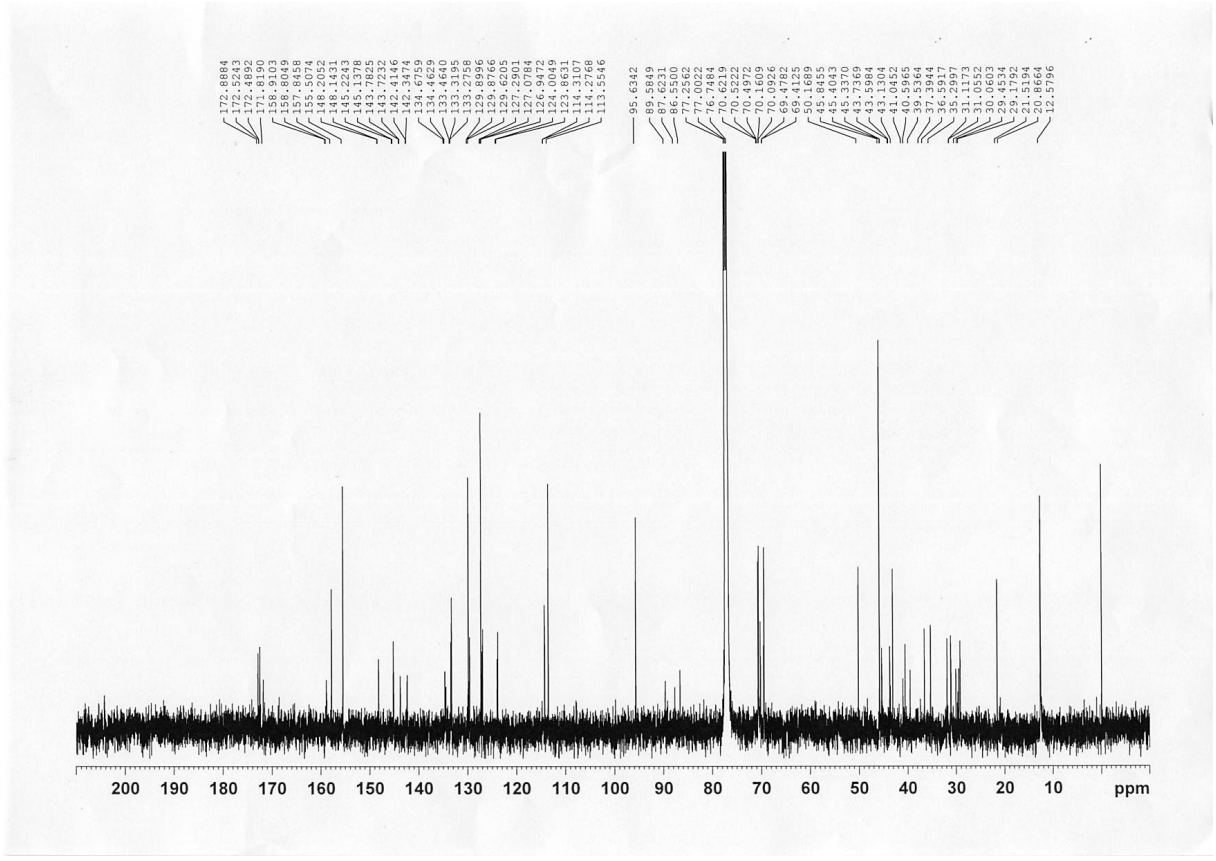
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4l** ($CDCl_3$)





^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **4m** ($CDCl_3$)





^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **5** (CD_3OD)