Supporting Information

Convergent synthesis of trifunctional molecules by three sequential azido-type-selective cycloadditions

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Contents

General Remarks

All reactions were performed in a dry glassware 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). Preparative thin-layer chromatography (PTLC) was performed on silica-gel (Wako Pure Chemical Industries Ltd., Wakogel® B-5F, Cat. No. 230-00043). Melting points (Mp) were measured on a YANACO MP-J3 instrument or an OptiMelt MPA100 (Stanford Research Systems), and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker AVANCE 400 or 500 spectrometer at 400 or 500 MHz, respectively. ^{13}C NMR spectra were obtained with a Bruker AVANCE 500 at 126 MHz. $CDCl_3$ (Acros Organics, Cat. No. 368651000), DMSO-d_6 (CIL, Cat. No. DLM-10), or CD_3OD (Acros Organics, Cat. No. 321280100) 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 in $CDCl_3$) or the solvent peak (δ 3.31 for ^{1}H NMR in CD_3OD, δ 2.49 for ^{1}H NMR in DMSO-d_6, δ 77.0 for ^{13}C NMR in $CDCl_3$, and δ 49.2 for ^{13}C NMR in CD_3OD) as an internal reference with coupling constants (J) in hertz (Hz). The abbreviations s, d, t, q, sept, m, and br signify singlet, doublet, triplet, quartet, septet, 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^{+}) or negative electrospray ionization (ESI^{-}) conditions. Elemental analyses were carried out at the Center for Advanced Material Analysis (Ookayama) of Tokyo Institute of Technology. High-performance liquid chromatography (HPLC) was performed on a Shimadzu Prominence HPLC system (CBM-20A lite, LC-20AD × 2, DGU-20A3R, SUS316L, and CTO-20A) equipped with a Shimadzu SPD-20A UV/Vis detector. Fluorescence spectra (FL) were recorded on a JASCO FP-8500 spectrofluorophotometer (emission and excitation bandwidth, 1 nm) at 20 °C using a quartz cuvette (10 mm light path).

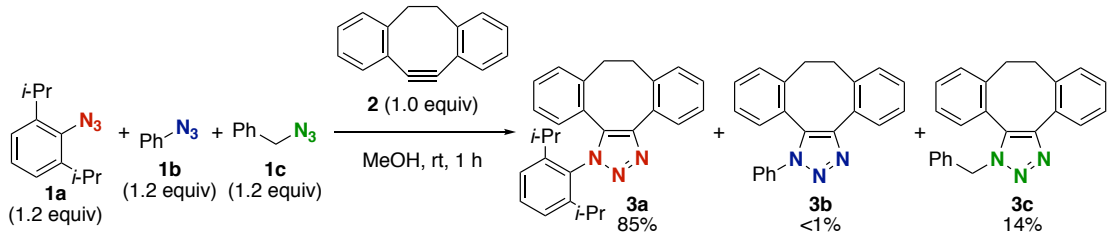
Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. benzyl azide (**1c**) (Cat. No. 327-79632), acetylacetone (**7**) (Cat. No. 013-00493), potassium carbonate (Cat. No. 162-03495), sodium azide (Cat. No. 197-11091), sodium nitrite (Cat. No. 196-02575), acetic acid (Cat. No. 017-00256), triethylamine (Cat. No. 202-02646), methanol (Cat. No. 137-01823), benzene (Cat. No. 024-00706), toluene (Cat. No. 204-01861), *N*,*N*-dimethylformamide (DMF) (Cat. No. 045-02911), dimethylsulfoxide (DMSO) (Cat. No. 043-07211), dichloromethane (Cat. No. 135-02441), 1,4-dioxane (Cat. No. 042-31655), tetrahydrofuran (THF) (Cat. No. 204-08745), and 2-butanol (Cat. No. 026-11212) were purchased from Wako Pure Chemical Industries Ltd. Tetrakis(acetonitrile)copper(I) tetrafluoroborate (Cat. No. T2666), diketene (stabilized with ethylene glycol) (Cat. No. K0002), ca. 25 wt % aqueous solution of tetramethylammonium hydroxide (Cat. No. T1460), *N*-benzylacetoacetamide (Cat. No. B2808), and 1,1,2,2-tetrachloroethane (Cat. No. T0063) were purchased from Tokyo Chemical Industry Co., Ltd. 2-Methyl-3-butyn-2-ol (**4b**) (Cat. No. 25500-30) and potassium acetate (Cat. No. 32299-30) were purchased from Kanto Chemical Co. Inc. Phenylacetylene (**4a**) (Cat. No. 117706), pentamethylcyclopentadienylbis(triphenylphosphine)ruthenium(II) chloride (Cat. No. 673293), dichlorobis(triphenylphosphine)-palladium(II) (Cat. No. 412740), and 11-azido-3,6,9-trioxaundecan-1-amine (Cat. No. 17758) were purchased from Sigma–Aldrich Japan. 5-((+)-Biotinamido)pentylamine (Cat. No. 21345) and (+)-biotinyl-3,6-dioxaoctanediamine (Cat. No. 21346) were purchased from Thermo Fisher Scientific K.K. Bis(pinacolato)diboron ($(Bpin)_2$) (Cat. No. AK-47583) was purchased from Ark Pharm, Inc.

2,6-Diisopropylphenyl azide (**1a**),[S1] phenyl azide (**1b**),[S2] 5,6-didehydro-11,12-dihydrodibenzo-[*a*,*e*]cyclooctene (**2**),[S3] 11,12-didehydro-5,6-dihydrodibenzo[*a,e*]cycloocten-5-yl 4-nitrophenyl carbonate,[S3] 4-iodo-2,6-diisopropylaniline,[S4] 1-azido-3-azidomethyl-5-iodobenzene (**9**),[S5] 2-(2-((6-chlorohexyl)oxy)-ethoxy)ethylamine,[S6] 2,5-dioxopyrrolidin-1-yl 4-ethynylbenzoate,[S7] [3-ethyl-5-[(4-ethyl-3,5-dimethyl-2*H*-pyrrol-2-ylidene-κN)(4-ethynylphenyl)methyl]-2,4-dimethyl-1*H*-pyrrolato-κN]difluoroboron (**14**),[S8] tris[(1-benzyl-1*H*-1,2,3-triazole-4-yl)methyl]amine (TBTA),[S9] and (1,3-dimesitylimidazol-2-ylidene)copper bromide[S10] were prepared according to the reported methods.

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.***

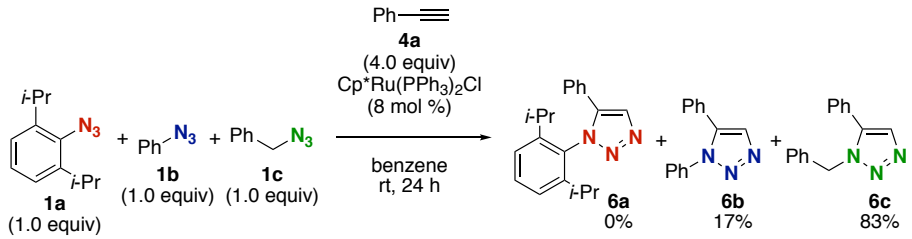
Chemical Experiments

*Competition reaction A: concerted cycloaddition with cyclooctyne **2** (Table 1, entry 1)*



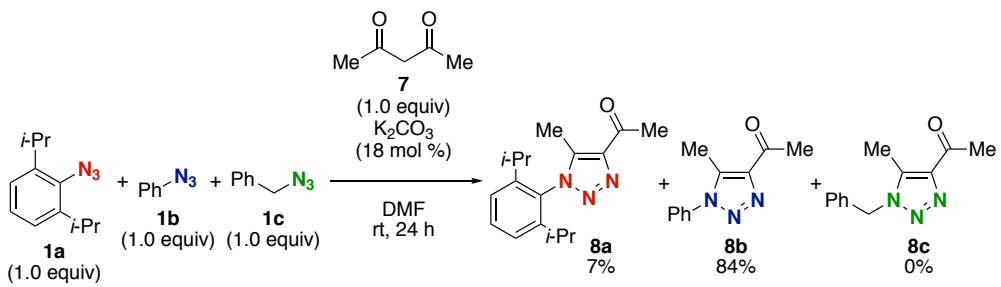
To a solution of 5,6-didehydro-11,12-dihydrodibenzo[*a,e*]cyclooctene (**2**) (20.4 mg, 99.9 μmol, 1.00 equiv) dissolved in methanol (11.0 mL) was added dropwise a mixture of 2,6-diisopropylphenyl azide (**1a**) (24.4 mg, 0.120 mmol, 1.20 equiv), phenyl azide (**1b**) (14.3 mg, 0.120 mmol, 1.20 equiv), and benzyl azide (**1c**) (16.0 mg, 0.120 mmol, 1.20 equiv) dissolved in methanol (1.5 mL) at room temperature. After stirring for 1 h at the same temperature, the mixture was concentrated under reduced pressure. To the residue was added 1,1,2,2-tetrachloroethane (14.9 mg, 88.8 μmol) as an internal standard, dissolved in $CDCl_3$, and ^1H NMR analysis (400 MHz) was performed. Yields of **3a**, **3b**, and **3c** were determined to be 85.2%, <1%, and 14.2%, respectively, by comparing the relative values of integration for the peaks observed at 7.67–7.68 ppm (m, 1H) for **3a**, 7.60–7.63 ppm (m, 1H) for **3b**, and 5.56 ppm (s, 2H) for **3c** with that of 1,1,2,2-tetrachloroethane observed at 5.95 ppm (s, 2H).

*Competition reaction B: ruthenium-catalyzed cycloaddition with terminal alkyne **4a** (Table 1, entry 4)*



To a solution of phenylacetylene (**4a**) (81.7 mg, 0.800 mmol, 4.00 equiv) dissolved in benzene (1.0 mL) was added pentamethylcyclopentadienylbis(triphenylphosphine)ruthenium(II) chloride (13.0 mg, 16.3 μmol, 8.2 mol %) at room temperature. After stirring for 5 min at the same temperature, to the solution was added a mixture of 2,6-diisopropylphenyl azide (**1a**) (40.7 mg, 0.200 mmol, 1.00 equiv), phenyl azide (**1b**) (23.8 mg, 0.200 mmol, 1.00 equiv), and benzyl azide (**1c**) (26.6 mg, 0.200 mmol, 1.00 equiv) dissolved in benzene (1.0 mL) at room temperature. After stirring for 24 h at the same temperature, the mixture was filtered through a short pad of silica gel (100 mg) and the filtrate was concentrated under reduced pressure. To the residue was added 1,1,2,2-tetrachloroethane (16.1 mg, 95.9 μmol) as an internal standard, dissolved in $CDCl_3$, and ^1H NMR analysis (400 MHz) was performed. Yields of **6a**, **6b**, and **6c** were determined to be 0%, 16.7%, and 83.4%, respectively, by comparing the relative values of integration for the peaks observed at 7.85 ppm (s, 1H) for **6b** and 5.52 ppm (s, 2H) for **6c** with that of 1,1,2,2-tetrachloroethane observed at 5.95 ppm (s, 2H).

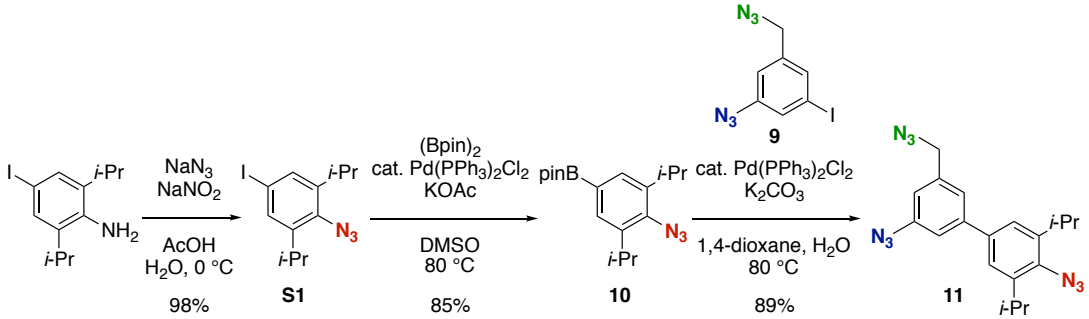
*Competition reaction C: base-catalyzed cycloaddition with 1,3-diketone **7** (Table 1, entry 6)*



To a mixture of 2,6-diisopropylphenyl azide (**1a**) (48.8 mg, 0.240 mmol, 1.00 equiv), phenyl azide (**1b**) (28.6 mg, 0.240 mmol, 1.00 equiv), benzyl azide (**1c**) (32.0 mg, 0.240 mmol, 1.00 equiv), and acetylacetone (**7**) (24.0 mg, 0.240 mmol, 1.00 equiv) dissolved in DMF (1.0 mL) was added potassium carbonate (6.0 mg,

43 μmol, 18 mol %) at room temperature. After stirring for 24 h at the same temperature, to the mixture was added water (10 mL). The mixture was extracted with EtOAc (20 mL × 3), and the combined organic extract was washed with water (10 mL × 3), brine (5 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. To the residue was added 1,1,2,2-tetrachloroethane (15.9 mg, 94.5 μmol) as an internal standard, dissolved in $CDCl_3$, and ^1H NMR analysis (400 MHz) was performed. Yields of **3a**, **3b**, and **3c** were determined to be 7.3%, 84.2%, and 0%, respectively, by comparing the relative values of integration for the peaks observed at 2.58 ppm (s, 3H) for **8a** and 2.34 ppm (s, 3H) for **8b** with that of 1,1,2,2-tetrachloroethane observed at 5.95 ppm (s, 2H).

*Synthesis of triazide **11** (Fig. 2A)*

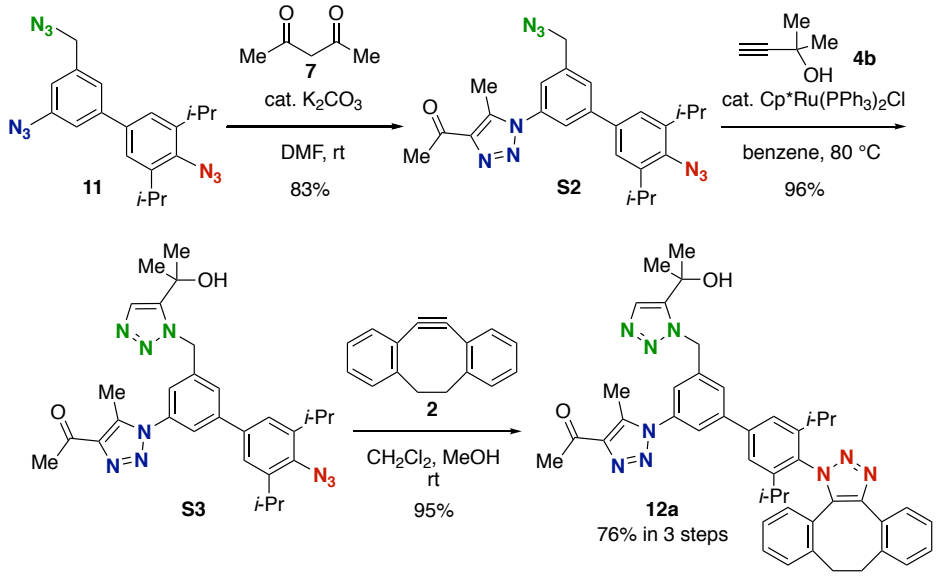


To a mixture of 4-iodo-2,6-diisopropylaniline (1.00 g, 3.30 mmol, 1.00 equiv) and sodium azide (429 mg, 6.60 mmol, 2.00 equiv) in acetic acid (20 mL) and distilled water (8.0 mL) was slowly added sodium nitrite (410 mg, 5.94 mmol, 1.80 equiv) at 0 °C. After stirring for 2 h at the same temperature, to the mixture was added a saturated aqueous solution of $NaHCO_3$ until the mixture became pH 10. The mixture was extracted with EtOAc (50 mL × 3), and the combined organic extract was washed with water (40 mL × 2) and brine (20 mL), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 30 g, *n*-hexane only) to give 4-iodo-2,6-diisopropylphenyl azide (**S1**) (1.06 g, 3.23 mmol, 97.9%) as a pale brown oil.

To a solution of 4-iodo-2,6-diisopropylphenyl azide (**S1**) (2.00 g, 6.08 mmol, 1.00 equiv) dissolved in DMSO (50 mL) were added bis(pinacolato)diboron (2.22 g, 8.74 mmol, 1.44 equiv), potassium acetate (1.77 g, 18.8 mmol, 3.09 equiv), and dichlorobis(triphenylphosphine)palladium(II) (220 mg, 0.313 mmol, 5.1 mol %) at room temperature and the mixture was heated at 80 °C with stirring for 2.5 h. After cooling to room temperature, to the mixture was added water (100 mL). The mixture was extracted with EtOAc (100 mL × 3), and the combined organic extract was washed with water (50 mL) and brine (20 mL × 2), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 50 g, *n*-hexane/EtOAc = 30/1 to 10/1) to give 2-(4-azido-3,5-diisopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**10**) (1.69 g, 5.14 mmol, 84.6%) as a pale brown oil.

To a solution of 1-azido-3-azidomethyl-5-iodobenzene (**9**) (150 mg, 0.500 mmol, 1.00 equiv) dissolved in 1,4-dioxane (2.5 mL) and water (0.5 mL) were added 2-(4-azido-3,5-diisopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**10**) (247 mg, 0.750 mmol, 1.50 equiv), potassium carbonate (140 mg, 1.01 mmol, 2.02 equiv), dichlorobis(triphenylphosphine)palladium(II) (18.0 mg, 25.6 μmol, 5.1 mol %) at room temperature and the mixture was heated at 80 °C with stirring for 2 h. After cooling to room temperature, to the mixture was added water (20 mL). The mixture was extracted with EtOAc (20 mL × 3), and the combined organic extract was washed with water (10 mL) and brine (5 mL × 2), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 10 g, *n*-hexane only) to give 3′,4-diazido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl (**11**) (167 mg, 0.444 mmol, 88.8%) as a pale brown oil.

*Three sequential cycloadditions of triazide **11** with three different azidophiles (1): 1) base-catalyzed cycloaddition, 2) ruthenium-catalyzed cycloaddition, and 3) strain-promoted cycloaddition (Fig. 2B upper scheme)*

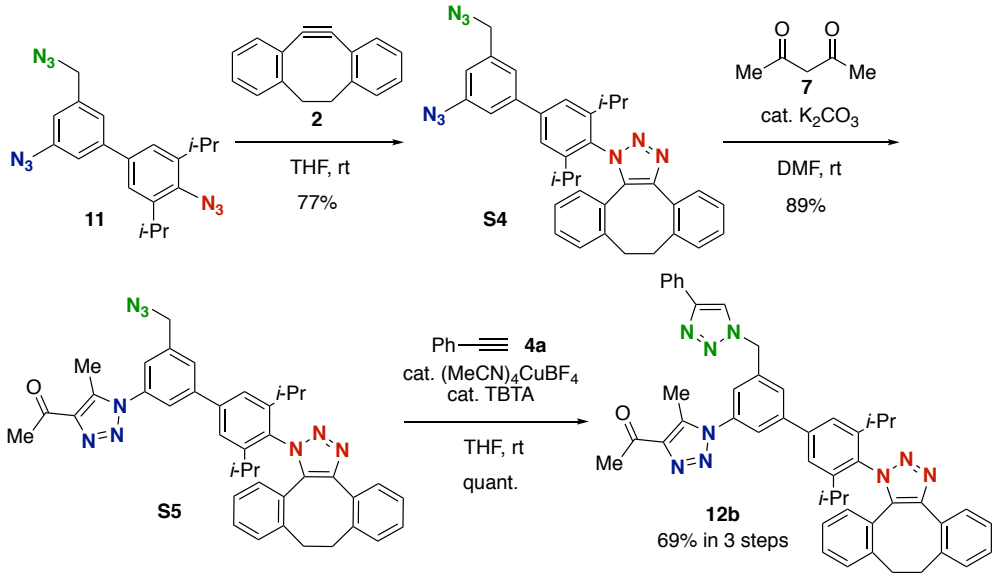


To a solution of of 3,4′-diazido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl (**11**) (80.0 mg, 0.213 mmol, 1.00 equiv) dissolved in DMF (2.1 mL) were added acetylacetone (**7**) (25.6 mg, 0.256 mmol, 1.20 equiv) and potassium carbonate (5.9 mg, 43 µmol, 20 mol %) at room temperature. After stirring for 24 h at the same temperature, to the mixture was added water (10 mL). The mixture was extracted with EtOAc (20 mL × 3), and the combined organic extract was washed with water (10 mL × 3), brine (5 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, *n*-hexane/EtOAc = 10/1 to 3/1) to give 1-(1-(4′-azido-5-azidomethyl-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S2**) (80.6 mg, 0.176 mmol, 82.7%) as a pale yellow solid.

To a solution of 1-(1-(4′-azido-5-azidomethyl-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S2**) (34.1 mg, 74.5 µmol, 1.00 equiv) dissolved in benzene (0.80 mL) were added 2-methyl-3-butyn-2-ol (**4b**) (9.4 mg, 0.12 mmol, 1.6 equiv) and (pentamethylcyclopentadieny)bis(triphenylphosphine)ruthenium(II) chloride (3.0 mg, 3.8 µmol, 5.1 mol %) at room temperature and the mixture was heated at 80 °C with stirring for 2 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, *n*-hexane only; *n*-hexane/EtOAc = 3/1 to 1/1; EtOAc only) to give 1-(1-(4′-azido-5-((5-(2-hydroxypropan-2-yl)-1*H*-1,2,3-triazol-1-ylmethyl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S3**) (38.7 mg, 71.4 µmol, 95.9%) as a pale yellow solid.

To a solution of 1-(1-(4′-azido-5-((5-(2-hydroxypropan-2-yl)-1*H*-1,2,3-triazol-1-ylmethyl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S3**) (15.3 mg, 28.2 µmol, 1.00 equiv) dissolved in dichloromethane (0.50 mL) and methanol (0.50 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a,e*]cyclooctene (**2**) (6.9 mg, 34 µmol, 1.2 equiv) at room temperature. After stirring for 2 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, *n*-hexane/EtOAc = 3/1 to 1/1; EtOAc only) to give 1-(1-(4′-(8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazol-1-yl)-5-((5-(2-hydroxypropan-2-yl)-1*H*-1,2,3-triazol-1-yl)methyl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**12a**) (20.0 mg, 26.8 µmol, 94.9%) as a colorless solid.

*Three sequential cycloadditions of triazide **11** with three different azidophiles (2):*
1) strain-promoted cycloaddition, 2) base-catalyzed cycloaddition, and 3) copper-catalyzed cycloaddition (Fig. 2B lower scheme)



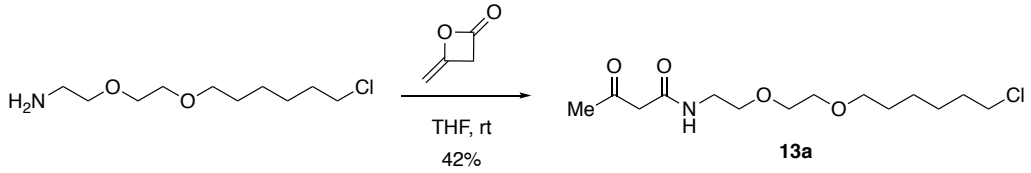
To a solution of 3,4′-diazido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl (**11**) (113 mg, 0.301 mmol, 3.01 equiv) dissolved in THF (6.5 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a,e*]cyclooctene (**2**) (20.4 mg, 99.9 µmol, 1.00 equiv) at room temperature. After stirring for 8 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (*n*-hexane/EtOAc = 3/1) to remove the remaining **11**, followed by flash column chromatography (silica-gel 5 g, *n*-hexane/EtOAc = 10/1 to 3/1) to give 1-(3′-azido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl-4-yl)-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**S4**) (44.3 mg, 76.4 µmol, 76.5%) as a colorless solid.

To a solution of of 1-(3′-azido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl-4-yl)-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**S4**) (18.2 mg, 31.4 µmol, 1.00 equiv) dissolved in DMF (0.30 mL) were added acetylacetone (**7**) (3.8 mg, 38 µmol, 1.2 equiv) and potassium carbonate (1.0 mg, 7.2 µmol, 23 mol %) at room temperature. After stirring for 24 h at the same temperature, to the mixture was added water (5 mL). The mixture was extracted with EtOAc (10 mL × 3), and the combined organic extract was washed with water (5 mL × 3), brine (5 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, *n*-hexane/EtOAc = 10/1 to 1/1) to give 1-(5-azidomethyl-4′-(8,9-dihydro-1*H*-dibenzo[3,4:7,8]cyclo-octa[1,2-*d*][1,2,3]triazol-1-yl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S5**) (18.5 mg, 28.0 µmol, 89.0%) as a colorless solid.

To a solution of 1-(5-azidomethyl-4′-(8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazol-1-yl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S5**) (6.0 mg, 9.1 µmol, 1.0 equiv) dissolved in THF (3.0 mL) were added phenylacetylene (**4a**) (1.5 mg, 15 µmol, 1.6 equiv), TBTA (1.0 mg, 1.9 µmol, 21 mol %), and tetrakis(acetonitrile)copper(I) tetrafluoroborate (1.0 mg, 3.2 µmol, 35 mol %) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (*n*-hexane/EtOAc = 1/1) to give 1-(1-(4′-(8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazol-1-yl)-3',5'-diisopropyl-5-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-1,1'-biphenyl)-3-yl-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**12b**) (6.9 mg, 9.0 µmol, quant.) as a colorless solid.

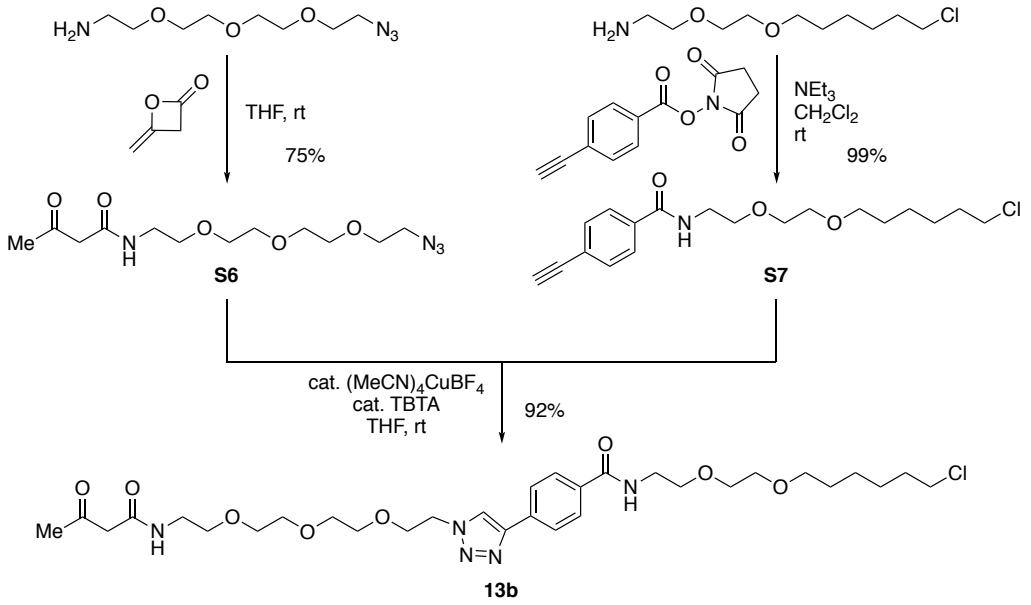
Preparation of functional modules used for the synthesis of trifunctional probe candidates ***16a–d***

Preparation of β-ketoamide ***13a*** *bearing a HaloTag ligand moiety*



To a solution of 2-(2-((6-chlorohexyl)oxy)ethoxy)ethylamine, preprared from *N-tert*-butyl (2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)carbamate (218 mg, 663 μmol, 1.00 equiv), dissolved in THF (5.0 mL) was added diketene (111 mg, 1.33 mmol, 2.01 equiv) at room temperature. After stirring for 12 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (CH_2Cl_2/MeOH = 10/1) to give *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-3-oxobutanamide (**13a**) (85.4 mg, 0.277 mmol, 41.9%) as a pale yellow oil.

Preparation of β-ketoamide ***13b*** *bearing a HaloTag-ligand moiety conjugated with a 3,6,9-trioxaundecamethylene (PEO₃) linker and a 1,2,3-triazole skeleton*



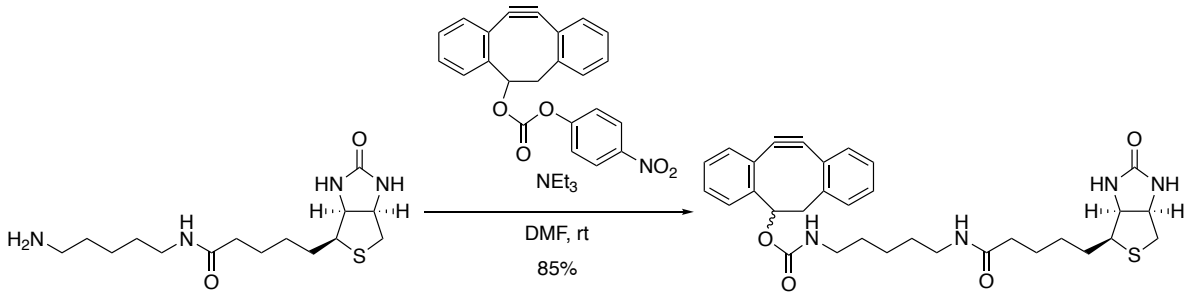
To a solution of diketene (38.5 mg, 0.458 mmol, 1.00 equiv) dissolved in THF (3.0 mL) was added 11-azido-3,6,9-trioxaundecan-1-amine (100 mg, 0.458 mmol, 1.00 equiv) at room temperature. After stirring for 11 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 10 g, CH_2Cl_2/MeOH = 30/1 to 10/1) to give *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-3-oxobutanamide (**S6**) containing a small amount of impurity. Further purification by recycling preparative HPLC system (JAI, LC-9210) equipped with a refractive index detector and JAIGEL-1H and 2H columns (GPC) using $CHCl_3$ as an eluent afforded *N*-(2-(2-(2-(2-azidoethoxy)-ethoxy)ethoxy)ethyl)-3-oxobutanamide (**S6**) (104 mg, 0.345 mmol, 75.0%) as a pale yellow oil.

To a solution of 2-(2-((6-chlorohexyl)oxy)ethoxy)ethylamine, preprared from *N-tert*-butyl (2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)carbamate (99.8 mg, 0.308 mmol, 1.07 equiv), dissolved in dichloromethane (3.0 mL) were added triethylamine (87.8 mg, 0.868 mmol, 3.01 equiv) and 2,5-dioxopyrrolidin-1-yl 4-ethynylbenzoate (70 .0 mg, 0.288 mmol, 1.00 equiv) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 10 g, *n*-hexane/EtOAc = 2/1 to 1/1) to give *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)-ethyl)-4-ethynylbenzamide (**S7**) (101 mg, 0.286 mmol, 99.3%) as a colorless solid.

To a solution of *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-3-oxobutanamide (**S6**) (50.0 mg, 0.165 mmol, 1.00 equiv) dissolved in THF (0.50 mL) were added *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-4-ethynylbenzamide (**S7**) (58.8 mg, 0.167 mmol, 1.01 equiv), tetrakis(acetonitrile)copper(I) tetrafluoroborate (2.6 mg, 8.3 μmol, 5.0 mol %), and TBTA (4.4 mg, 8.3 μmol, 5.0 mol %) at room temperature. After stirring

for 48 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, CH_2Cl_2 only; then CH_2Cl_2/MeOH = 30/1) to give *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-4-(1-(13,15-dioxo-3,6,9-trioxa-12-azahexadecyl)-1*H*-1,2,3-triazol-4-yl)benzamide (**13b**) (100 mg, 0.153 mmol, 92.4%) as a colorless solid.

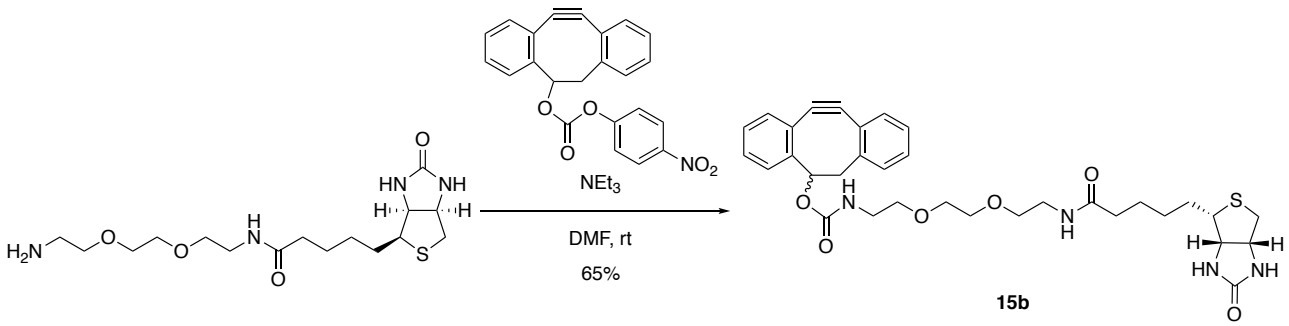
*Preparation of cyclooctyne **15a** bearing a biotin moiety conjugated with a pentamethylene linker*



15a

To a solution of 5-((+)-biotinamido)pentylamine (46.2 mg, 99.9 μmol, 1.00 equiv) dissolved in DMF (10 mL) were added 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl 4-nitrophenyl carbonate (46.2 mg, 0.120 mmol, 1.20 equiv) and triethylamine (30.4 mg, 0.300 mmol, 3.00 equiv) at room temperature. After stirring for 24 h at the same temperature, to the mixture was added 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl 4-nitrophenyl carbonate (18.0 mg, 54.8 μmol, 0.549 equiv) again at room temperature. After stirring for 4 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, CH_2Cl_2 only; then CH_2Cl_2/MeOH = 30/1 to 10/1) to give cyclooctyne **15a** (48.9 mg, 85.1 μmol, 85.2%) as a colorless solid.

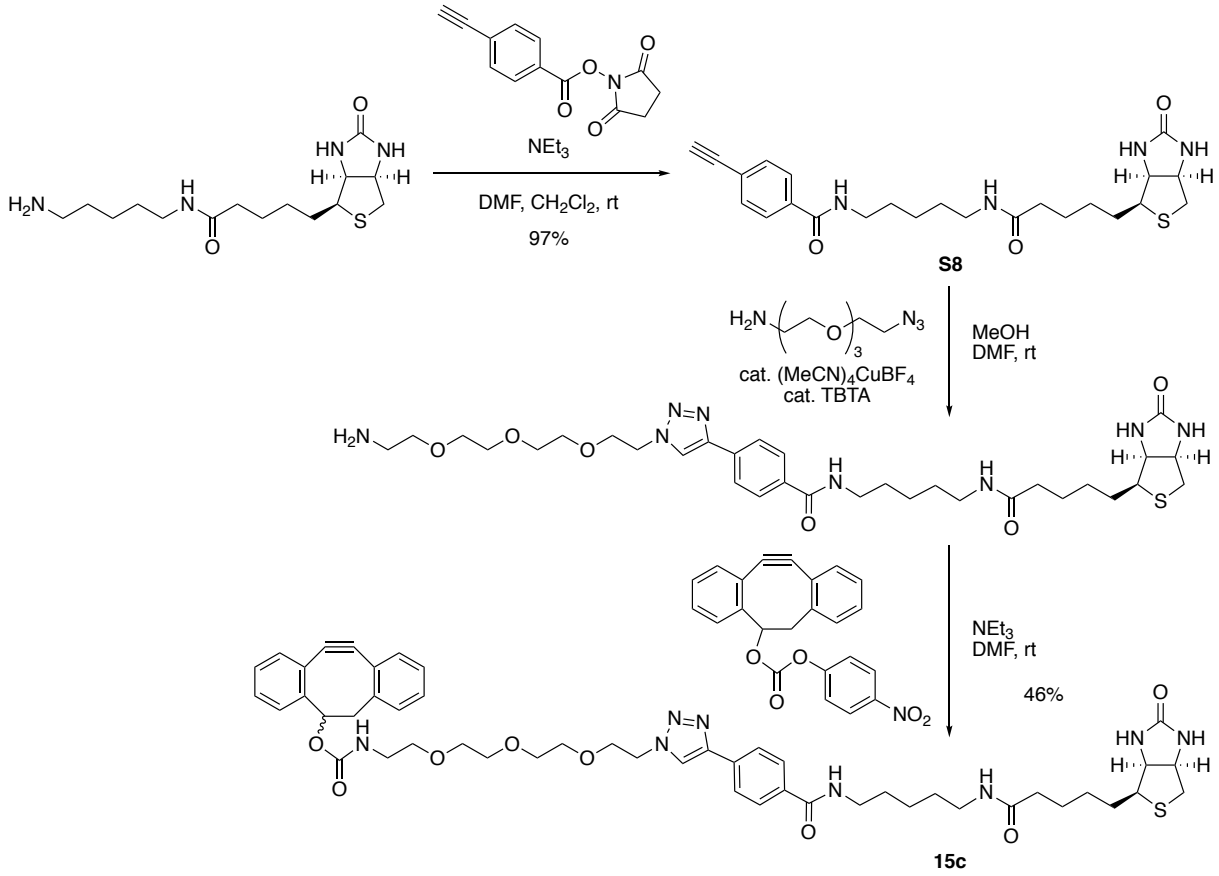
*Preparation of cyclooctyne **15b** bearing a biotin moiety conjugated with a 3,6-dioxaoctamethylene (PEO_2) linker*



15b

To a solution of 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl 4-nitrophenyl carbonate (25.9 mg, 67.2 μmol, 1.20 equiv) dissolved in DMF (5.0 mL) were added (+)-biotinyl-3,6-dioxaoctanediamine (21.0 mg, 56.1 μmol, 1.00 equiv) and triethylamine (15.2 mg, 0.150 mmol, 2.67 equiv) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, CH_2Cl_2/MeOH = 20/1 to 6/1) to give cyclooctyne **15b** (22.7 mg, 36.6 μmol, 65.2%) as a colorless solid.

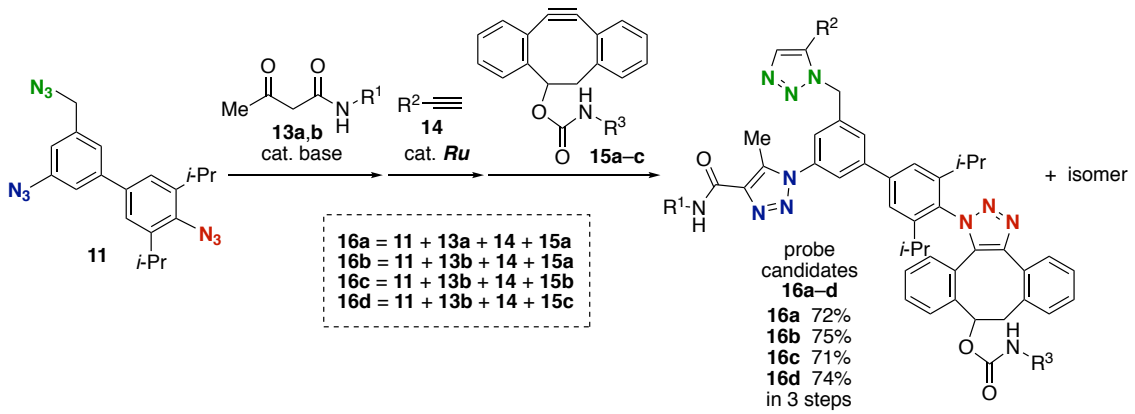
*Preparation of cyclooctyne **15c** bearing a biotin moiety conjugated with 3,6,9-trioxaundecamethylene (PEO_3) and pentamethylene linkers linked by a 1,2,3-triazole skeleton*



To a solution of 5-((+)-biotinamido)pentylamine (20.0 mg, 60.9 μmol, 1.00 equiv) dissolved in dichloromethane (1.0 mL) and DMF (1.0 mL) were added 2,5-dioxopyrrolidin-1-yl 4-ethynylbenzoate (17.8 mg, 73.0 μmol, 1.20 equiv) and triethylamine (18.2 mg, 0.180 mmol, 2.96 equiv) at room temperature. After stirring for 9 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, CH_2Cl_2/MeOH = 10/1 to MeOH only) to give 4-ethynyl-*N*-(5-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)pentyl)benzamide (**S8**) (25.4 mg, 59.3 μmol, 97.3%) as a colorless solid.

To a solution of 4-ethynyl-*N*-(5-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-pentanamido)pentyl)benzamide (**S8**) (25.4 mg, 59.3 μmol, 1.01 equiv) dissolved in MeOH (1.5 mL) and DMF (0.20 mL) were added 11-azido-3,6,9-trioxaundecan-1-amine (12.8 mg, 58.6 μmol, 1.00 equiv), TBTA (3.2 mg, 6.0 μmol, 10 mol %), and tetrakis(acetonitrile)copper(I) tetrafluoroborate (1.9 mg, 6.0 μmol, 10 mol %) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was dissolved in DMF (2.0 mL) and to the solution were added 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl 4-nitrophenyl carbonate (38.5 mg, 99.9 μmol, 1.70 equiv) and triethylamine (18.2 mg, 0.180 mmol, 3.07 equiv) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, CH_2Cl_2/MeOH = 10/1 to MeOH only) to give cyclooctyne **15c** (25.0 mg, 27.1 μmol, 46.3%) as a colorless solid.

*Synthesis of trifunctional probe candidates **16a–d***



*General procedures for triazole formation reactions conducted for the synthesis of trifunctional probe candidates **16a–d***

*General procedure **A**: base-catalyzed cycloaddition at the sterically unhindered aromatic azido group of triazide **11** using a β-ketoamide*

To a solution of of 3,4′-diazido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl (**11**) (0.10 mmol) dissolved in DMF (1.0 mL) were added β-ketoamide **13a** or **13b** (0.12 mmol) and potassium carbonate (20 µmol) at room temperature. After stirring for 24 h at the same temperature, to the mixture was added water (10 mL). The mixture was extracted with EtOAc (20 mL × 3), and the combined organic extract was washed with water (10 mL × 3), brine (5 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography to give the corresponding triazole.

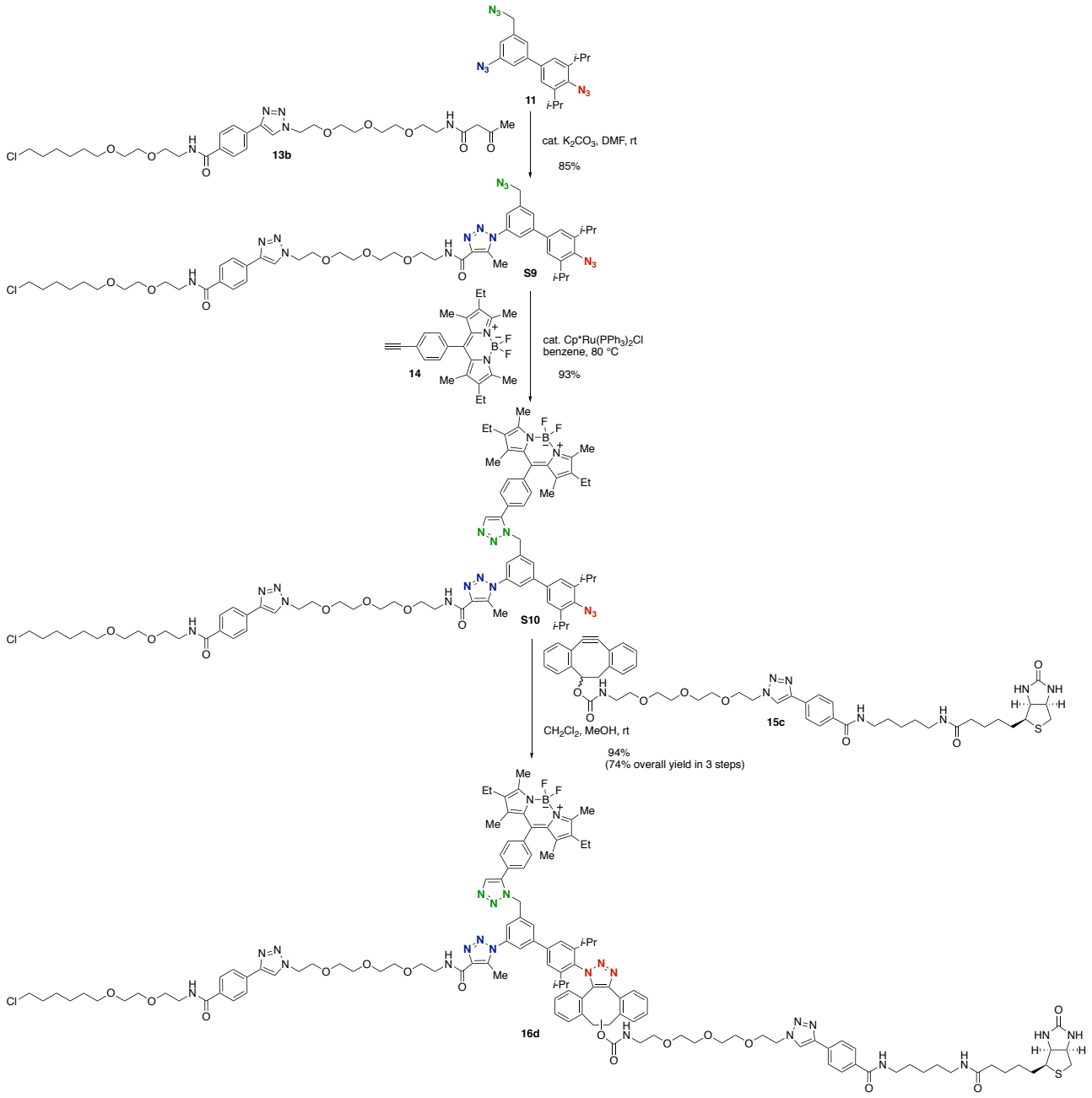
*General procedure **B**: Ru-catalyzed cycloaddition at the aliphatic azido group of a diazido intermediate with terminal alkyne **14***

To a solution of diazide (75 µmol) dissolved in toluene (0.80 mL) were added terminal alkyne **14** (0.120 mmol) and (pentamethylcyclopentadieny)bis(triphenylphosphine)ruthenium(II) chloride (4 µmol) at room temperature and the mixture was heated at 80 °C with stirring for 2 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography to give the corresponding bistriazole.

*General procedure **C**: strain-promoted cycloaddition with a cyclooctyne*

To a solution of azide (20 µmol) dissolved in dichloromethane (0.50 mL) and methanol (0.50 mL) was added cyclooctyne **15a**, **15b**, or **15c** (22 µmol) at room temperature. After stirring for 4 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography to give the corresponding tristriazole.

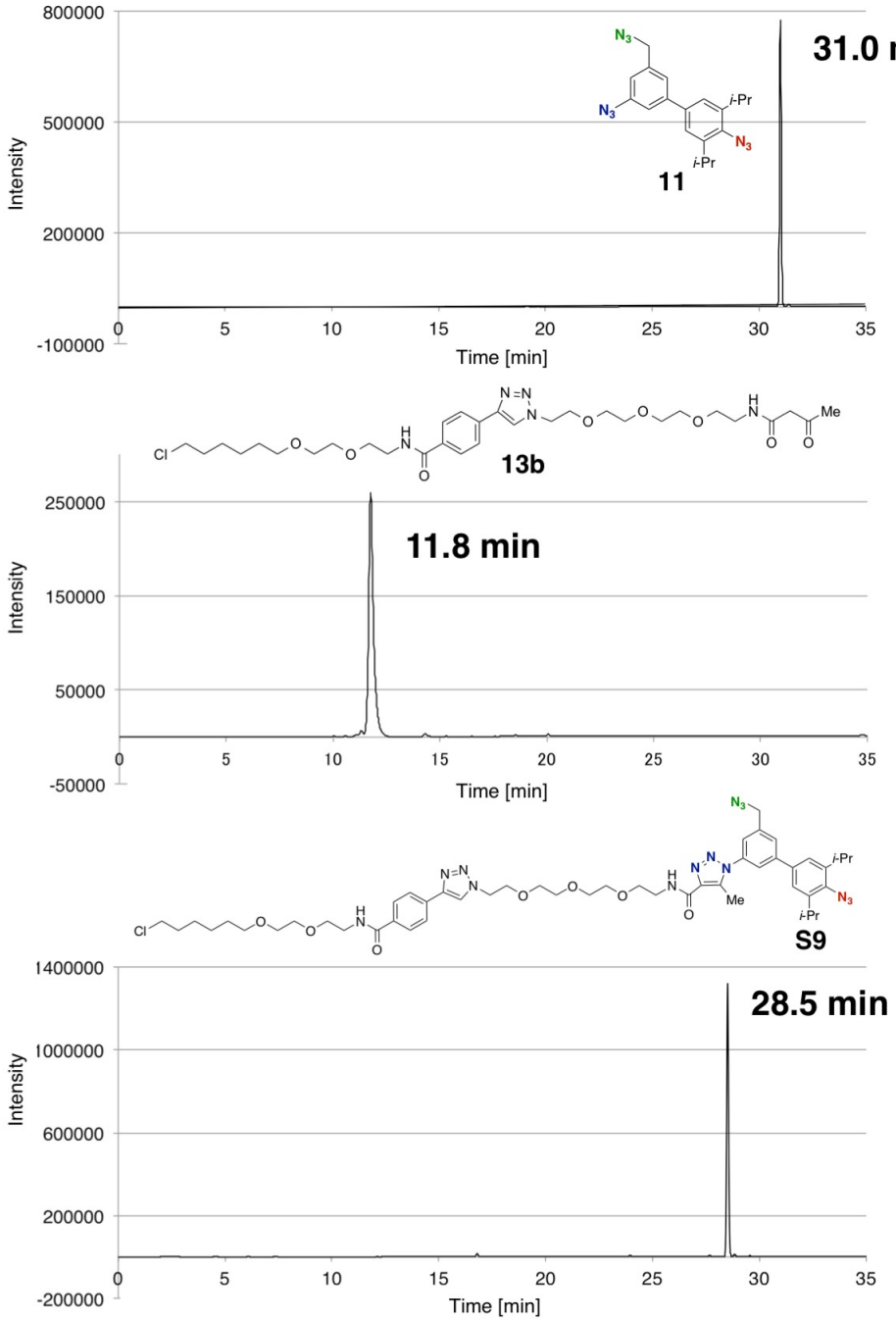
Synthesis of trifunctional probe ***16d*** *(****11*** *+* ***13b*** *+* ***14*** *+* ***15c****)*



Since NMR analysis of these compounds gave complex spectra, the purity of each product was confirmed by analytical reverse phase HPLC [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm].

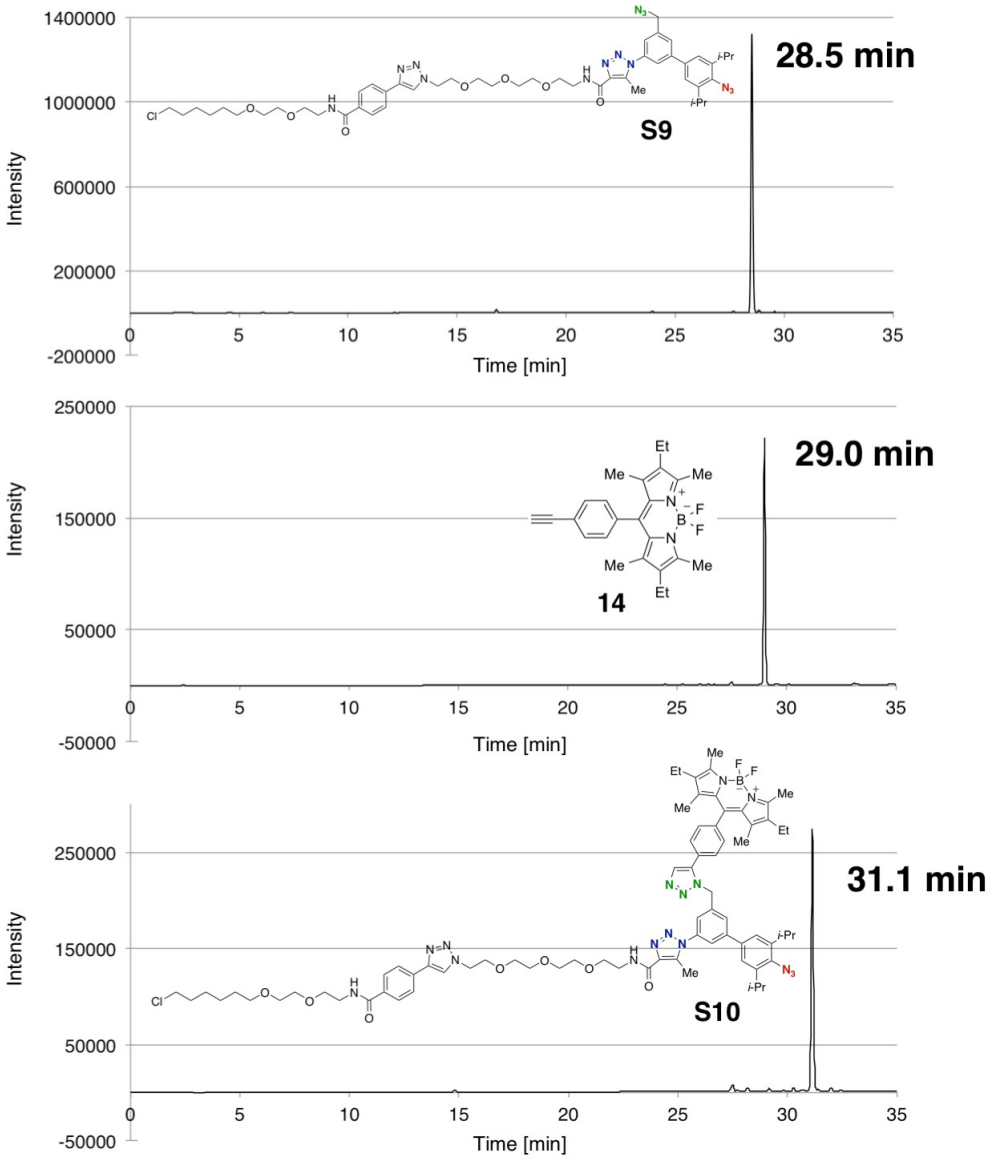
*Base-catalyzed cycloaddition at the aromatic azido group of triazide **11** using β-ketoamide **13b***

To a solution of of 3′,4-diazido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl (**11**) (21.0 mg, 55.9 μmol, 1.00 equiv) dissolved in DMF (1.0 mL) were added *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-4-(1-(13,15-dioxo-3,6,9-trioxa-12-azahexadecyl)-1*H*-1,2,3-triazol-4-yl)benzamide (**13b**) (44.0 mg, 67.3 μmol, 1.20 equiv) and potassium carbonate (1.4 mg, 10 μmol, 18 mol %) at room temperature. After stirring for 24 h at the same temperature, to the mixture was added water (10 mL). The mixture was extracted with EtOAc (20 mL × 3), and the combined organic extract was washed with water (10 mL × 3), brine (5 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, CH_2Cl_2/MeOH = 50/1 to 30/1) to give diazide **S9** (48.3 mg, 47.7 μmol, 85.4%) as a pale orange oil.



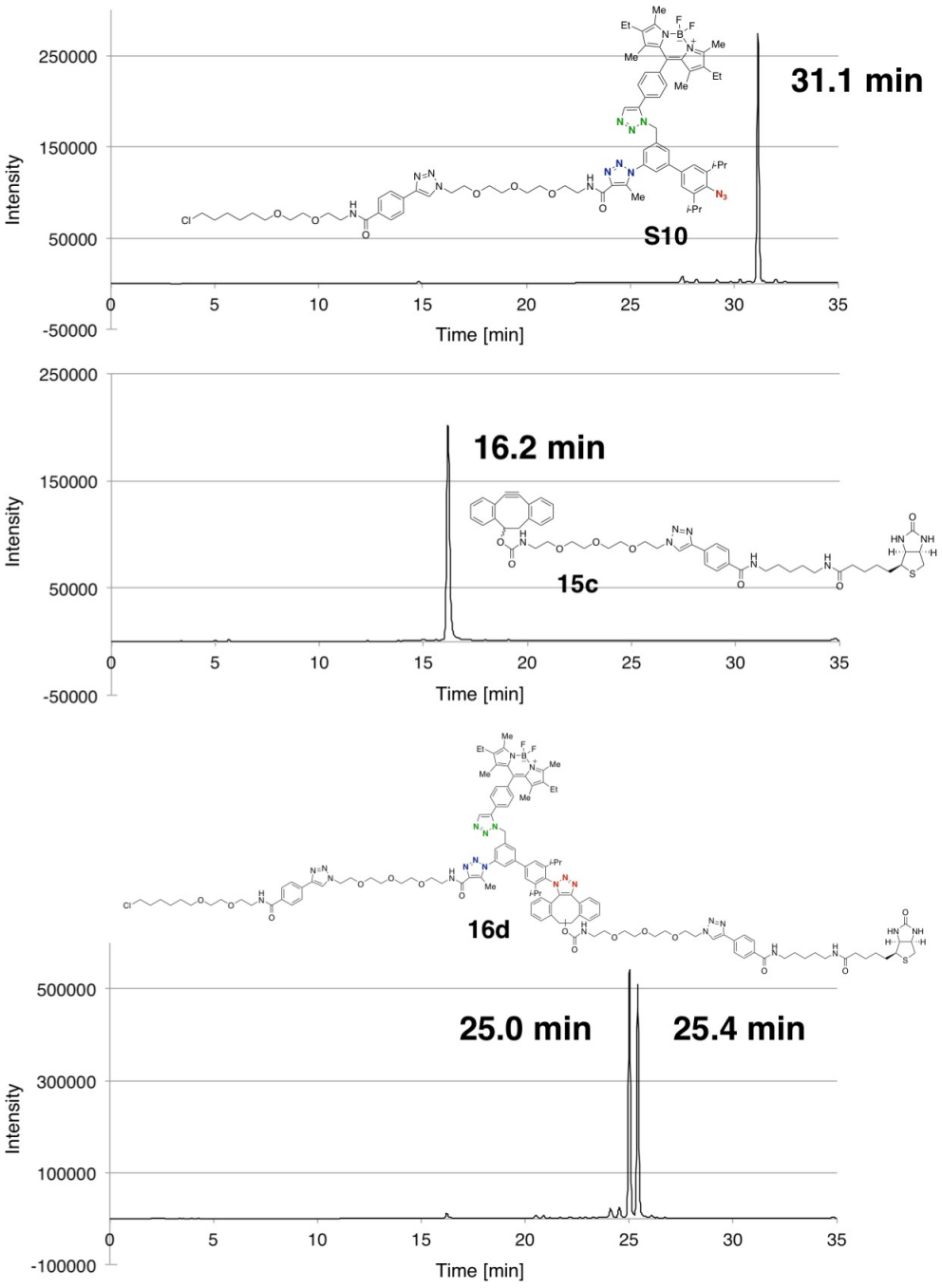
*Ru-catalyzed cycloaddition at the aliphatic azido group of diazide **S9** with terminal alkyne **14***

To a solution of diazide **S9** (31.6 mg, 31.2 µmol, 1.00 equiv) dissolved in toluene (0.30 mL) were added [3-ethyl-5-[(4-ethyl-3,5-dimethyl-2*H*-pyrrol-2-ylidene-*κN*)(4-ethynylphenyl)methyl]-2,4-dimethyl-1*H*-pyrrolato-*κN*]difluoroboron (**14**) (18.0 mg, 44.5 µmol, 1.43 equiv) and (pentamethylcyclopentadieny)bis-(triphenylphosphine)ruthenium(II) chloride (1.1 mg, 2.9 µmol, 9.3 mol %) at room temperature and the mixture was heated at 80 °C with stirring for 2 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 5 g, CH_2Cl_2 only; then CH_2Cl_2/MeOH = 10/1) to give bistriazole **S10** (41.0 mg, 29.0 µmol, 92.7%) as a pink solid.



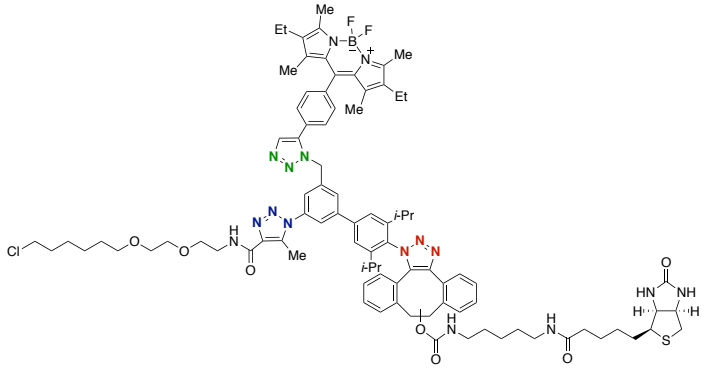
*Strain-promoted cycloaddition of azide **S10** with cyclooctyne **15c***

To a solution of azide **S10** (12.0 mg, 8.48 µmol, 1.1 equiv) dissolved in dichloromethane (0.50 mL) and methanol (0.50 mL) was added cyclooctyne **15c** (5.0 mg, 8.1 µmol, 1.0 equiv) at room temperature. After stirring for 2 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 2 g, CH_2Cl_2 only; then CH_2Cl_2/MeOH = 20/1 to 5/1) to give tristriazole **16d** (15.5 mg, 7.6 µmol, 94%) as a pink solid.

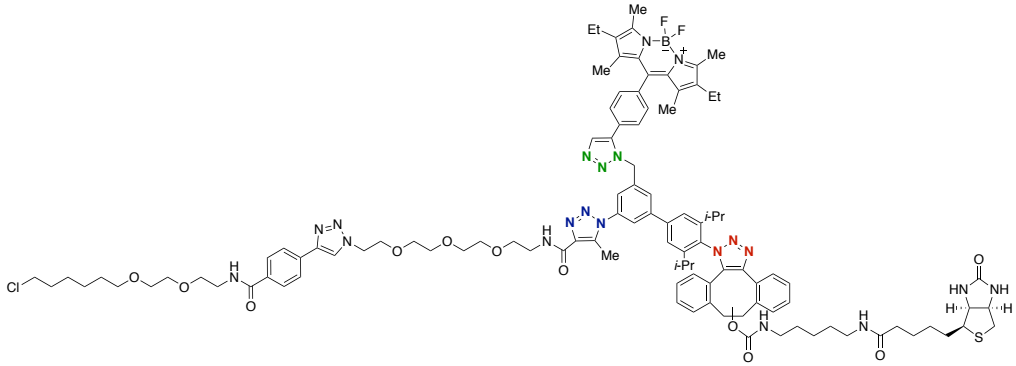


Similarly, trifunctional probe candidates **16a–c** and three kinds of bisfunctional tristriazoles **S11–S13** for a control experiment were prepared from triazide **11** according to the general procedures **A–C**.

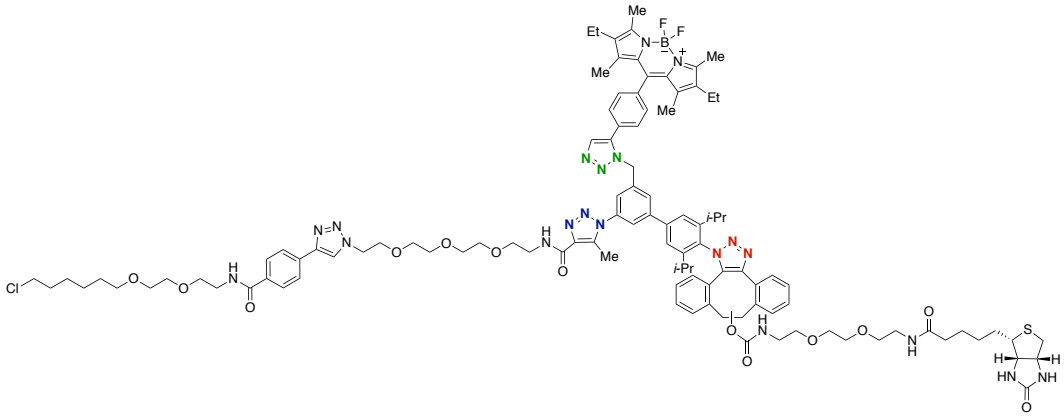
16a: **11** + **13a** + **14** + **15a**: 72% overall yield in 3 steps.



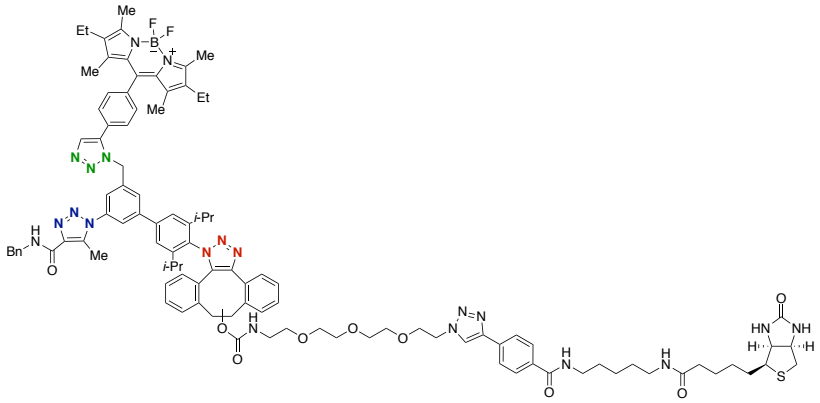
16b: **11** + **13b** + **14** + **15a**: 75% overall yield in 3 steps.



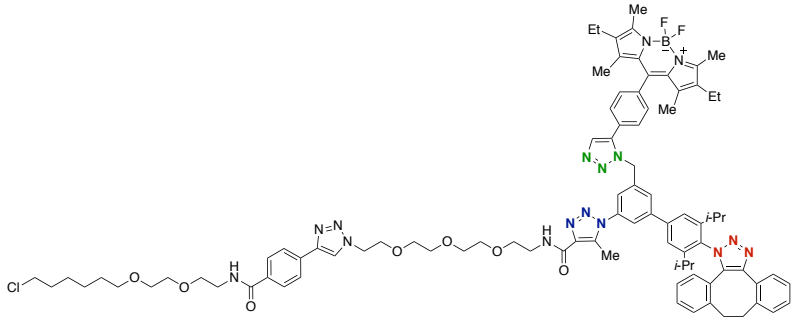
16c: **11** + **13b** + **14** + **15b**: 71% overall yield in 3 steps.



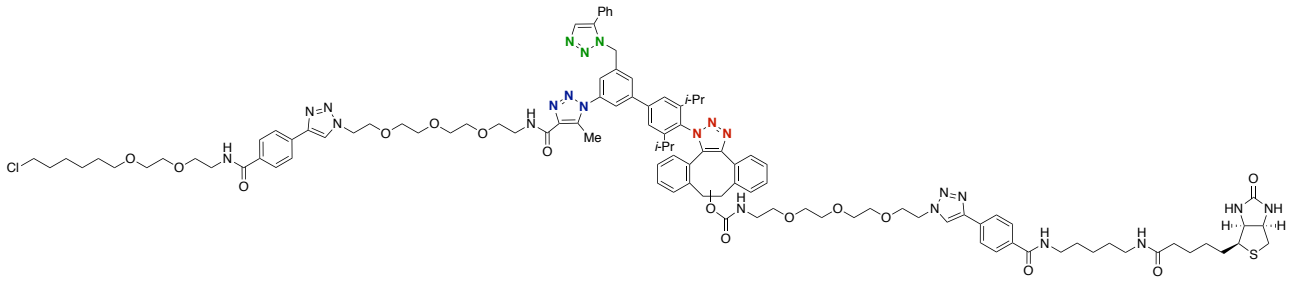
Bisfunctional tristriazole **S11** without the HaloTag ligand moiety:
11 + *N*-benzylacetoacetamide + **14** + **15c**



Bisfunctional tristriazole **S12** without the biotin moiety: **11** + **13b** + **14** + **2**



Bisfunctional tristriazole **S13** without the BODIPY moiety: **11** + **13b** + **4a** + **15c**



Biological Experiments

Production of recombinant GST-HaloTag protein in *E. coli*.

Escherichia coli strain Rosetta (DE3) pLysS cells (Millipore, Merck Chemicals Ltd., Nottingham, England) were transformed with pGEX6P-1-HaloTag vector,[S11] and cultured in LB media containing 50 $mg \cdot L^{-1}$ carbenicillin (Nacalai Tesque, Kyoto, Japan) and 34 $mg \cdot L^{-1}$ chloramphenicol (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Expression was induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG) (final concentration at 0.2 mM) (Nacalai Tesque), when the culture had reached an OD_{600} of approximately 0.8. At the same time, the culture without IPTG was also prepared. After induction for 16 h at 37 °C, the cells were collected by centrifugation at 5000 *g* for 10 min, and suspended in phosphate buffered saline (PBS). The suspended cells were frozen in liquid N_2, and stored at –80 ºC until use. After thawing on ice, Triton X-100 (final concentration at 1%*)* was added to the cell lysate, which were then incubated on ice for 20 min. $MgCl_2$ (final concentration at 2 mM) and DNase I (final concentration of approximately 10 $\mu g \cdot mL^{-1}$) were added to the cell lysate, and incubation was continued at 4 °C for 20 min with gentle agitation. Cell debris and larger particles were removed by centrifugation at 12,000 *g* for 30 min, and the supernatant was then filtered through a 0.45-μm filter. The filtrated supernatants were frozen in liquid N_2, and stored at –80 ºC until use for the following labeling experiments.

Chemical modification of the HaloTag protein in the crude extracts.

Into five hundred microlitter of the filtrated supernatants in a 1.5 mL-tube, five microlitter of the indicated compounds (10 mM stock in DMSO) were added, and immediately mix by vortex for 10 sec to be the final concentration of 100 μM. The solvent DMSO was used as a negative control. The mixtures were rotated gently in a dark room at room temperature for 16 h. Twenty-five microlitter of this reacted mixtures was diluted with equal volume of 2× SDS sample loading buffer (0.12 M Tris-HCl, pH 6.8, containing 3.4% SDS, 10% glycerol, and 20 mM DTT; Nacalai tesque), heated at 98 °C for 10 min.

SDS-PAGE analysis was carried out under reducing conditions using a 5–10% gradient separation gel (ATTO Corporation, Tokyo, Japan). The gels were directly visualized by laser-scanning with excitation by 488-nm wavelength of the blue laser followed by detecting emission light through 520-nm band-pass filter (520 BP 40) in a fluorescence imaging analyzer Typhoon 9410 (GE Healthcare), and then stained with Coomassie brilliant blue (CBB) EzStain AQua (ATTO Corporation). Images of the stained gels were taken by a flathead scanner GT-X970 (Seiko Epson Corporation, Japan).

The separated proteins in the gels were electrically transferred onto PVDF membranes in Mini Trans-Blot Cell (Bio-Rad Laboratories, Inc., Japan). The membranes were immersed in Blocking One solution (Nacalai Tesque), and then incubated with horseradish peroxidase-conjugated streptavidin (HRP-streptavidin) (Kirkegaard & Perry Laboratories, Meryland, USA) diluted in 1% Blocking One/Tris-based saline containing 0.1% Tween 20 (TBST) at 4 ºC for 16 h. The membranes were extensively washed with TBST, and then reacted with ECL Western Blotting Detection Reagents (GE Healthcare). Luminescence signals were imaged on ChemiDox XRS Imaging System (Bio-Rad).

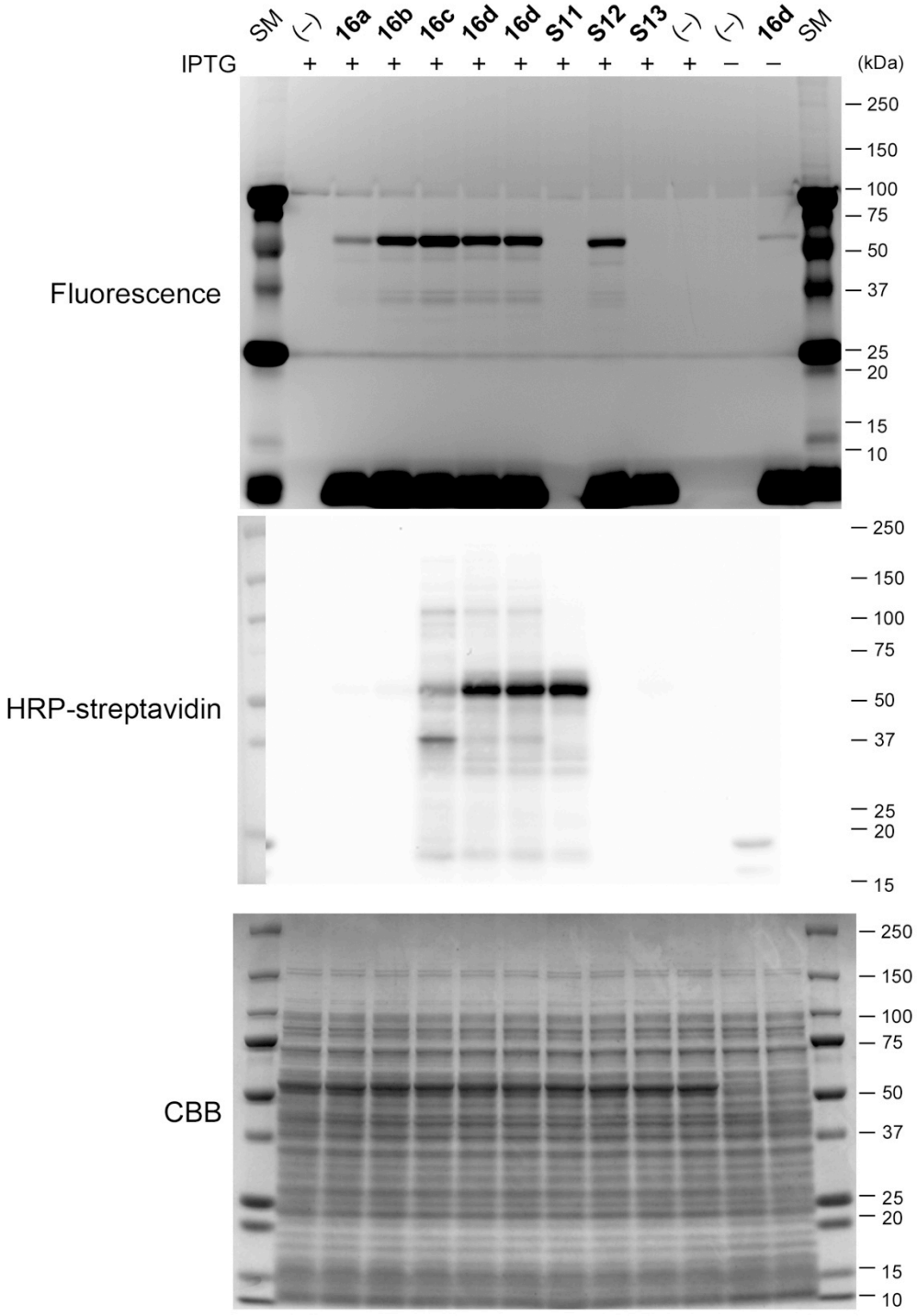


Figure S1. *Chemical modification of the HaloTag protein by probe candidates* ***16a–d*** *and* ***S11–S13****.*

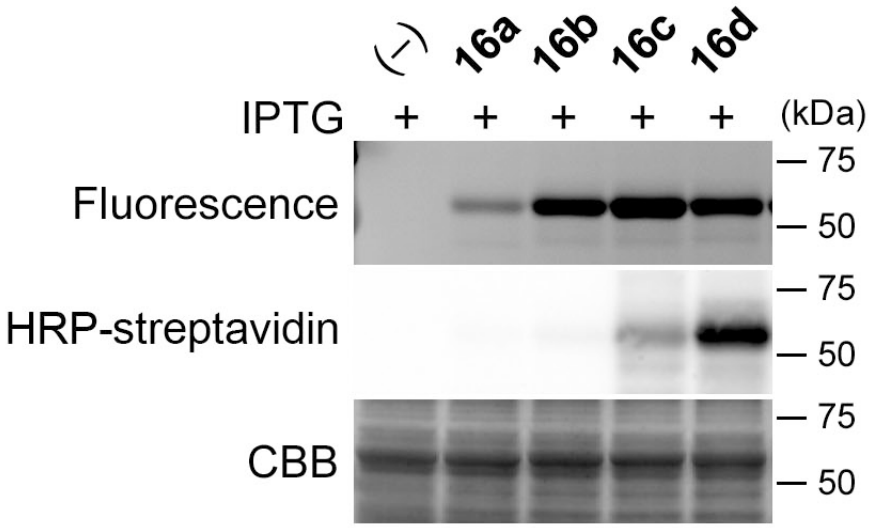


Figure S2. *Chemical modification of the HaloTag protein by trifunctional probe candidates* ***16a–d*** *(focused Figure S1).*

The results clearly show that each function of trifunctional probe **16d**, chemical modification of HaloTag protein (59 kDa) and detection of fluorescence and biotin, worked efficiently as expected. On the other hand, in the case of using **S11** without the HaloTag ligand moiety, neither fluorescence nor biotin was detected. When **S12** without the biotin moiety was used for the chemical modification, fluorescent labeling of HaloTag protein was achieved, but detection by HRP-streptavidin failed. Chemical modification by **S13** without the BODIPY moiety also resulted in a single-labeling; although modified HaloTag protein was detected by using HRP-streptavidin, fluorescence was not detected.

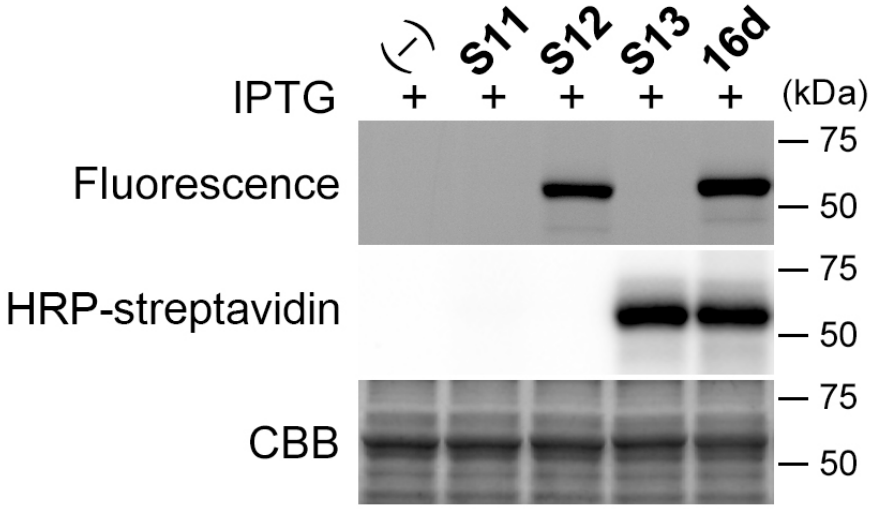
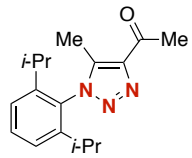


Figure S3. *Chemical modification of the HaloTag protein by trifunctional probe candidate* ***16d*** *and bisfunctional tristriazoles* ***S11–S13*** *(focused Figure S1).*

Characterization Data of New Compounds

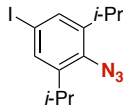
1-(2,6-Diisopropylphenyl)-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**3a**),[S12] 1-phenyl-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**3b**),[S12] 1-benzyl-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**3c**),[S13] 1-(2,6-diisopropylphenyl)-4-phenyl-1*H*-[1,2,3]-triazole (**5a**),[S14] 1,4-diphenyl-1*H*-[1,2,3]triazole (**5b**),[S15] 1-benzyl-4-phenyl-1*H*-[1,2,3]triazole (**5c**),[S16] 1-(2,6-diisopropylphenyl)-5-phenyl-1*H*-[1,2,3]triazole (**6a**),[S12] 1,5-diphenyl-1*H*-[1,2,3]triazole (**6b**),[S12] 1-benzyl-4-phenyl-1*H*-[1,2,3]triazole (**6c**),[S17] 1-(1-phenyl-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**8b**),[S18] and 1-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**8c**)[S19] showed identical spectra with those reported in the literature.

1-(1-(2,6-Diisopropylphenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**8a**)



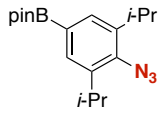
Colorless solid; Mp 115–118 °C; TLC R_f = 0.42 (*n*-hexane/EtOAc = 5/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.12 (d, 6H, *J* = 7.5 Hz), 1.13 (d, 6H, *J* = 7.5 Hz), 2.07 (tt, 2H, *J* = 7.5, 7.5 Hz), 2.35 (s, 3H), 2.80 (s, 3H), 7.33 (d, 2H, *J* = 7.5 Hz), 7.54 (t, 1H, *J* = 7.5 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 9.63 (1C), 23.1 (2C), 24.6 (2C), 27.7 (1C), 28.5 (2C), 124.2 (2C), 130.5 (1C), 131.3 (1C), 139.0 (1C), 143.0 (1C), 146.2 (2C), 194.6 (1C); IR (KBr, cm^{-1}) 763, 952, 1275, 1369, 1477, 1556, 1685, 2967; HRMS (ESI^+) *m/z* 308.1729 ($[M+Na]^+$, $C_{17}H_{23}N_3NaO^+$ requires 308.1733).

4-Iodo-2,6-diisopropylphenyl azide (**S1**)



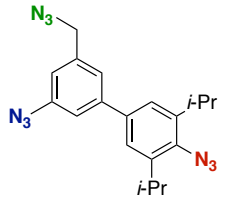
Pale brown oil; TLC R_f= 0.65 (*n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (d, 12H, *J* = 6.8 Hz), 3.28 (sept, 2H, *J* = 6.8 Hz), 7.42 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 23.3 (4C), 28.7 (2C), 92.3 (1C), 133.2 (2C), 135.3 (1C), 145.5 (2C); IR (KBr, cm^{-1}) 865, 1071, 1234, 1332, 1438, 1565, 2102, 2124, 2930, 2964; Anal. calcd. for $C_{12}H_{16}IN_3$: C, 43.78; H, 4.90; N, 12.77%; Found: C, 43.61; H, 5.20; N, 12.53%.

2-(4-Azido-3,5-diisopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**10**)



Pale brown oil; TLC R_f = 0.53 (*n*-hexane/EtOAc = 10/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (d, 12H, *J* = 6.8 Hz), 1.34 (s, 12H), 3.35 (sept, 2H, *J* = 6.8 Hz), 7.57 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 23.5 (4C), 24.8 (4C), 28.8 (2C), 83.8 (2C), 130.6 (2C), 138.0 (1C), 142.2 (2C). The signal for the carbon which is attached to the boron atom was not observed; IR (KBr, cm^{-1}) 692, 852, 966, 1146, 1378, 1463, 1601, 2117, 2933, 2967; Anal. calcd. for $C_{18}H_{28}BN_3O_2$: C, 65.66; H, 8.57; N, 12.76%; Found: C, 65.67; H, 8.86; N, 12.83%.

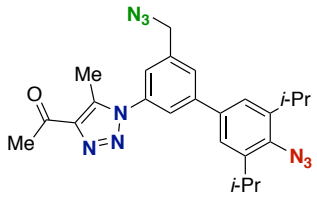
3′,4-Diazido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl (**11**)



Pale brown oil; TLC R_f = 0.14 (*n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (d, 12H), 3.41 (sept, 2H, *J* = 6.8 Hz), 4.42 (s, 2H), 6.98 (dd, 1H, *J* = 1.6, 1.6 Hz), 7.13 (dd, 1H, *J* = 1.6, 1.6 Hz), 7.23 (s, 1H), 7.28 (s, 2H);

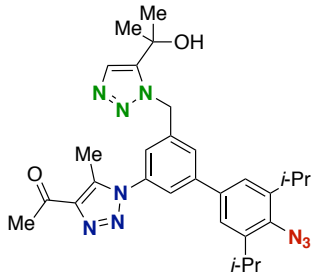
^{13}C NMR (126 MHz, $CDCl_3$) δ 23.5 (4C), 28.9 (2C), 54.3 (1C), 117.1 (1C), 117.7 (1C), 122.9 (2C), 123.4 (1C), 135.4 (1C), 137.9 (1C), 138.2 (1C), 141.1 (1C), 143.7 (1C), 143.7 (2C); IR (KBr, cm^{-1}) 852, 1292, 1335, 1439, 1593, 2108, 2871, 2966; Anal. calcd. for $C_{19}H_{21}N_9$: C, 60.78; H, 5.64; N, 33.58%; Found: C, 61.02; H, 5.94; N, 33.42%.

1-(1-(4′-Azido-5-azidomethyl-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S2**)



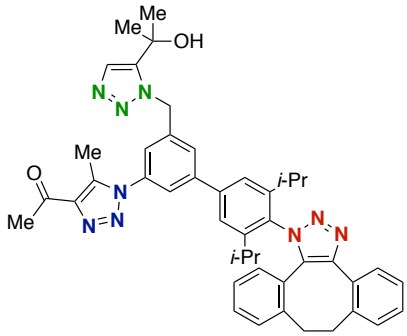
Pale yellow solid; Mp 116–118 °C; TLC R_f = 0.40 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, 12H, J = 6.8 Hz), 2.65 (s, 3H), 2.78 (s, 3H), 3.43 (sept, 2H, J = 6.8 Hz), 4.57 (s, 2H), 7.32 (s, 2H), 7.39 (dd, 1H, J = 1.2, 1.2 Hz), 7.58 (dd, 1H, J = 1.2, 1.2 Hz), 7.65 (dd, 1H, J = 1.2, 1.2 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 10.3 (1C), 23.5 (4C), 27.9 (1C), 28.9 (2C), 54.0 (1C), 123.0 (2C), 123.1 (1C), 123.8 (1C), 128.1 (1C), 135.8 (1C), 136.2 (1C), 137.3 (1C), 137.6 (1C), 138.1 (1C), 143.79 (1C), 143.84 (1C), 144.0 (2C), 194.4 (1C); IR (KBr, cm^{-1}) 866, 953, 1076, 1337, 1443, 1557, 1597, 1682, 2101, 2965, 3368; Anal. calcd. for $C_{24}H_{27}N_9O$: C, 63.00; H, 5.95; N, 27.55%; Found: C, 62.96; H, 5.68; N, 27.33%.

1-(1-(4′-Azido-5-((5-(2-hydroxypropan-2-yl)-1*H*-1,2,3-triazol-1-ylmethyl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S3**)



Pale yellow solid; Mp 78 °C (dec.); TLC R_f = 0.47 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (d, 12H, J = 6.8 Hz), 1.63 (s, 6H), 2.55 (s, 3H), 2.65–2.69 (br, 1H), 2.73 (s, 3H), 3.41 (sept, 2H, J = 6.8 Hz), 5.99 (s, 2H), 7.18 (s, 1H), 7.28 (s, 2H), 7.45 (s, 1H), 7.53 (s, 1H), 7.65 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 10.2 (1C), 23.5 (4C), 27.8 (1C), 28.9 (2C), 30.9 (2C), 52.1 (1C), 68.0 (1C), 122.7 (1C), 122.9 (2C), 123.4 (1C), 127.8 (1C), 130.9 (1C), 135.7 (1C), 135.9 (1C), 137.2 (1C), 137.5 (1C), 138.9 (1C), 143.1 (1C), 143.5 (2C), 143.6 (1C), 143.9 (1C), 194.2 (1C); IR (KBr, cm^{-1}) 732, 1175, 1464, 1558, 1683, 2123, 2966, 3322; Anal. calcd. for $C_{29}H_{35}N_9O_2$: C, 64.31; H, 6.51; N, 23.27%; Found: C, 64.09; H, 6.78; N, 23.08%.

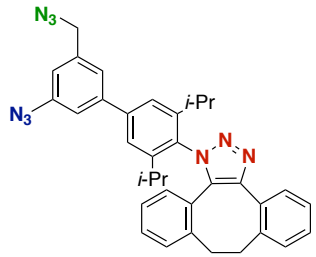
1-(1-(4′-(8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazol-1-yl)-5-((5-(2-hydroxypropan-2-yl)-1*H*-1,2,3-triazol-1-yl)methyl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**12a**)



Colorless solid; Mp 145 °C (dec); TLC R_f = 0.53 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.67–1.27 (br,

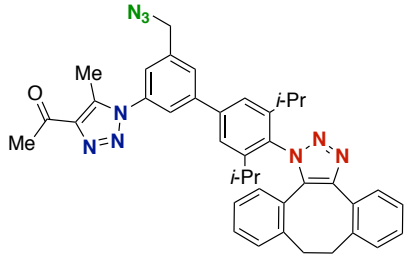
12H), 1.57 (s, 6H), 2.31–2.45 (br, 2H), 2.49 (s, 3H), 2.66 (s, 3H), 2.78 (s, 1H), 2.99–3.14 (br, 2H), 3.24–3.39 (br, 2H), 5.92 (s, 2H), 6.65 (dd, 1H, J = 0.8, 7.6 Hz), 6.90 (ddd, 1H, J = 0.8, 7.6, 7.6 Hz), 7.12–7.20 (m, 5H), 7.24–7.29 (m, 3H), 7.39 (s, 1H), 7.52 (dd, 1H, J = 1.6, 1.6 Hz), 7.59–7.61 (m, 1H), 7.65 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 10.2 (1C), 22.5 (2C), 25.4 (2C), 27.9 (1C), 28.9 (2C), 30.9 (2C), 32.7 (1C), 36.8 (1C), 52.0 (1C), 68.0 (1C), 122.9 (2C), 123.2 (1C), 123.8 (1C), 125.7 (1C), 126.0 (1C), 126.2 (1C), 128.0 (1C), 128.1 (1C+1C, two signals overlapped), 128.7 (1C), 129.4 (1C), 129.7(1C), 129.9 (1C), 130.91 (1C), 130.94 (1C), 132.3 (1C), 132.5 (1C), 135.1 (1C), 136.0 (1C), 137.4 (1C), 137.6 (1C), 139.1 (1C), 141.0 (1C), 141.4 (1C), 143.1 (2C), 143.7 (1C), 146.2 (1C), 147.3 (1C), 194.2 (1C); IR (KBr, cm^{-1}) 731, 910, 1096, 1368, 1557, 1682, 2965, 3300; HRMS (ESI^+) m/z 746.3901 ($[M+H]^+$, $C_{45}H_{48}N_9O_2^+$ requires 746.3925).

1-(3′-Azido-5′-azidomethyl-3,5-diisopropyl-1,1′-biphenyl-4-yl)-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**S4**)



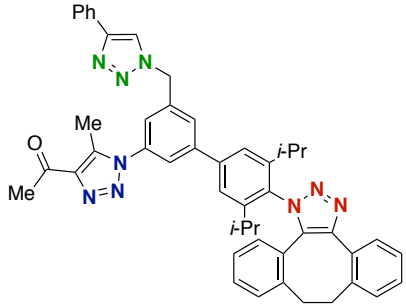
Colorless solid; Mp 155 °C (dec.); TLC R_f = 0.60 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78–1.34 (br, 12H), 2.39–2.53 (br, 2H), 3.12–3.21 (br, 2H), 3.35–3.51 (br, 2H), 4.44 (s, 2H), 6.74 (dd, 1H, J = 1.2, 8.0 Hz), 6.96 (ddd, 1H, J = 1.2, 7.6, 7.6 Hz), 7.02 (s, 1H), 7.16–7.35 (m, 9H), 7.68–7.71 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 22.6 (1C), 25.5 (2C), 28.9 (2C), 32.7 (2C), 36.9 (1C), 54.3 (1C), 117.6 (1C), 117.9 (1C), 122.9 (2C), 123.5 (1C), 125.8 (1C), 126.0 (1C+1C, two signals overlapped), 126.1 (1C), 128.1 (1C), 128.8 (1C), 129.6 (2C), 129.9 (1C), 130.9 (1C), 131.9 (1C), 132.6 (1C), 135.1 (1C), 137.4 (1C), 138.0 (1C), 141.2 (1C), 141.5 (1C), 141.9 (1C), 143.3 (1C), 146.2 (1C), 147.0 (br, 1C); IR (KBr, cm^{-1}) 732, 1274, 1354, 1454, 1597, 2108, 2871, 2931, 2965; Anal. calcd. for $C_{35}H_{33}N_9$: C, 72.52; H, 5.74; N, 21.75%; Found: C, 72.27; H, 6.03; N, 21.45%.

1-(5-Azidomethyl-4′-(8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazol-1-yl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**S5**)



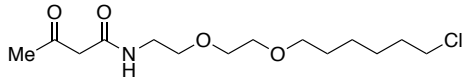
Colorless solid; Mp 218 °C (dec.); TLC R_f = 0.45 (*n*-hexane/EtOAc = 2/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84–1.36 (br, 12H), 2.42–2.55 (br, 2H), 2.67 (s, 3H), 2.78 (s, 3H), 3.09–3.23 (br, 2H), 3.33–3.46 (br, 2H), 4.58 (s, 2H), 6.73 (dd, 1H, J = 1.2, 7.6 Hz), 6.96 (ddd, 1H, J = 1.2, 7.6, 7.6 Hz), 7.20–7.42 (m, 8H), 7.64 (dd, 1H, J = 1.2, 1.2 Hz), 7.68–7.71 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 10.3 (1C), 22.5 (2C), 25.4 (2C), 27.9 (1C), 28.9 (2C), 32.7 (1C), 36.8 (1C), 54.0 (1C), 123.0 (2C), 123.5 (1C), 124.1 (1C), 125.8 (1C), 126.0 (1C), 126.1 (1C), 128.1 (1C), 128.3 (1C), 128.7 (1C), 129.5 (1C), 129.6 (1C), 129.9 (1C), 130.9 (2C), 132.4 (1C), 132.6 (1C), 135.1 (1C), 136.2 (1C), 137.4 (1C), 137.6 (1C), 138.2 (1C), 141.0 (1C), 141.5 (1C), 143.5 (1C), 143.8 (1C), 146.2 (1C), 147.4 (br, 1C), 194.3 (1C); IR (KBr, cm^{-1}) 733, 908, 1292, 1341, 1439, 1593, 2110, 2928, 2963; HRMS (ESI^+) m/z 662.3353 ($[M+H]^+$, $C_{40}H_{40}N_9O^+$ requires 662.3350).

1-(1-(4′-(8,9-Dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazol-1-yl)-3′,5′-diisopropyl-5-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-1,1′-biphenyl)-3-yl-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (**12b**)



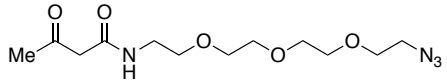
Colorless solid; Mp 144–146 °C; TLC R_f = 0.22 (*n*-hexane/EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85–1.34 (br, 12H), 2.41–2.53 (br, 2H), 2.61 (s, 3H), 2.76 (s, 3H), 3.11–3.23 (br, 2H), 3.36–3.45 (br, 2H), 5.78 (s, 2H), 6.72 (dd, 1H, *J* = 0.8, 7.6 Hz), 6.96 (ddd, 1H, *J* = 1.2, 7.6, 7.6 Hz), 7.19–7.44 (m, 11H), 7.65–7.72 (m, 3H), 7.81–7.84 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 10.3 (1C), 22.5 (2C), 25.5 (2C), 27.9 (1C), 28.9 (2C), 32.7 (1C), 36.8 (1C), 53.4 (1C), 119.7 (1C), 123.0 (2C), 123.4 (1C), 124.6 (1C), 125.69 (1C), 125.73 (2C), 126.0 (1C+1C, two signals overlapped), 126.1 (1C), 128.1 (1C), 128.2 (1C), 128.5 (1C), 128.7 (1C), 128.9 (2C), 129.4 (1C), 129.7 (1C), 129.9 (1C), 130.0 (1C), 130.9 (1C), 132.50 (1C), 132.55 (1C), 135.1 (1C), 136.5 (1C), 137.4 (1C), 137.48 (1C), 137.50 (1C), 140.7 (1C), 141.5 (1C), 143.8 (2C), 146.2 (1C), 147.4 (1C), 148.7 (1C), 194.2 (1C); IR (KBr, cm^{-1}) 763, 1074, 1366, 1464, 1557, 1682, 2962, 3054; HRMS (ESI^+) *m*/*z* 764.3792 ($[M+H]^+$, $C_{48}H_{46}N_9O^+$ requires 764.3820).

N-(2-(2-((6-Chlorohexyl)oxy)ethoxy)ethyl)-3-oxobutanamide (**13a**)



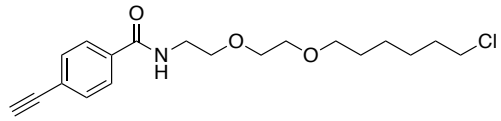
Pale yellow oil; TLC R_f = 0.25 (CH_2Cl_2/MeOH = 10/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41–1.48 (m, 4H), 1.58–1.65 (m, 2H), 1.75 (m, 2H), 2.27 (s, 3H), 3.40 (s, 2H), 3.46–3.63 (m, 12H), 7.06 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.4 (1C), 26.7 (1C), 29.4 (1C), 30.9 (1C), 32.5 (1C), 39.3 (1C), 45.0 (1C), 50.0 (1C), 69.6 (1C), 70.0 (1C), 70.4 (1C), 71.3 (1C), 165.5 (1C), 204.0 (1C); IR (KBr, cm^{-1}) 1119, 1358, 1547, 1649, 1719, 2862, 2936, 3308; HRMS (ESI^+) *m*/*z* 308.1626 ($[M+H]^+$, $C_{14}H_{27}{}^{35}ClNO_4^+$ requires 308.1623).

N-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-3-oxobutanamide (**S6**)



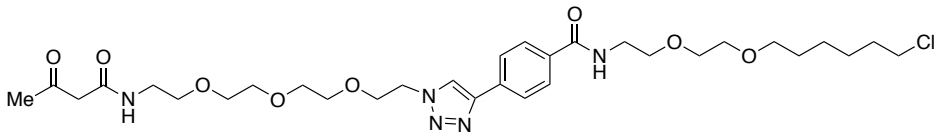
Pale yellow oil; TLC R_f = 0.70 (CH_2Cl_2/MeOH = 10/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 3H), 3.32–3.34 (m, 4H), 3.39–3.42 (m, 2H), 3.50–3.52 (m, 2H), 3.56–3.63 (m, 10H), 7.15 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 30.7 (1C), 39.2 (1C), 50.1 (1C), 50.6 (1C), 69.5 (1C), 69.9 (1C), 70.2 (1C), 70.49 (1C), 70.52 (1C), 70.6 (1C), 165.6 (1C), 203.9 (1C); IR (KBr, cm^{-1}) 1118, 1288, 1535, 1649, 1716, 2108, 2870, 3066, 3305; HRMS (ESI^-) *m*/*z* 301.1510 ($[M–H]^-$, $C_{12}H_{21}N_4O_5^-$ requires 301.1517).

N-(2-(2-((6-Chlorohexyl)oxy)ethoxy)ethyl)-4-ethynylbenzamide (**S7**)



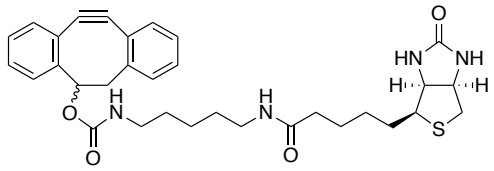
Colorless solid; TLC R_f = 0.27 (*n*-hexane/EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29–1.45 (m, 4H), 1.53–1.59 (m, 2H), 1.70–1.76 (m, 2H), 3.21 (s, 1H), 3.44–3.68 (m, 12H), 6.82 (br s, 1H), 7.53 (d, 2H, *J* = 8.0 Hz), 7.75 (d, 2H, *J* = 8.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.2 (1C), 26.5 (1C), 29.3 (1C), 32.3 (1C), 39.6 (1C), 44.9 (1C), 69.4 (1C), 69.8 (1C), 70.1 (1C), 71.1 (1C), 79.4 (1C), 82.6 (1C), 125.1 (1C), 126.9 (2C), 132.0 (2C), 134.4 (1C), 166.5 (1C); IR (KBr, cm^{-1}) 768, 855, 1113, 1303, 1538, 1642, 2862, 2937, 3290; HRMS (ESI^+) *m*/*z* 374.1490 ($[M+Na]^+$, $C_{19}H_{26}{}^{35}ClNNaO_3^+$ requires 374.1493).

N-(2-(2-((6-Chlorohexyl)oxy)ethoxy)ethyl)-4-(1-(13,15-dioxo-3,6,9-trioxa-12-azahexadecyl)-1*H*-1,2,3-triazol-4-yl)benzamide (**13b**)



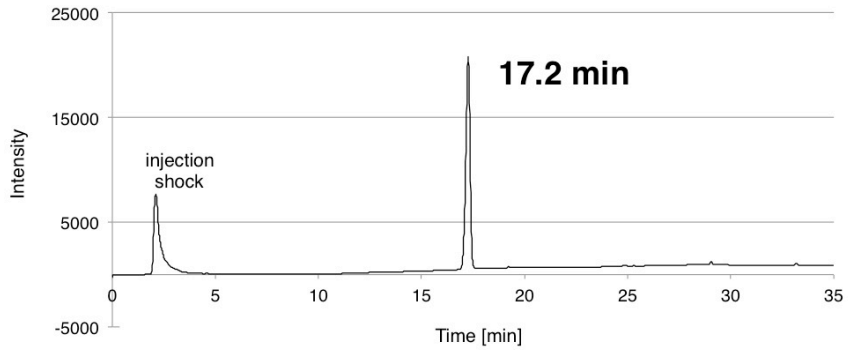
Colorless solid; Mp 79–81 °C; TLC R_f = 0.38 (CH_2Cl_2/MeOH = 10/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32–1.44 (m, 4H), 1.56–1.58 (m, 2H), 1.69–1.76 (m, 2H), 2.24 (s, 3H), 3.39–3.52 (m, 10H), 3.56–3.70 (m, 16H), 3.94 (t, 2H, *J* = 4.8 Hz), 4.62 (t, 2H, *J* = 4.8 H), 6.80–6.85 (br, 1H), 7.14–7.22 (br, 1H), 7.85 (d, 2H, *J* = 8.4 Hz), 7.92 (d, 2H, *J* = 8.4 Hz), 8.09 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.4 (1C), 26.7 (1C), 29.4 (1C), 30.9 (1C), 32.5 (1C), 39.2 (1C), 39.7 (1C), 45.0 (1C), 50.0 (1C), 50.4 (1C), 69.4 (1C), 69.6 (1C), 69.7 (1C), 70.0 (1C), 70.2 (1C), 70.3 (1C), 70.5 (1C), 70.5 (1C), 70.6 (1C), 71.3 (1C), 121.7 (1C), 125.6 (2C), 127.7 (2C), 133.7 (1C), 133.9 (1C), 146.7 (1C), 165.7 (1C), 167.0 (1C), 204.2 (1C); IR (KBr, cm^{-1}) 1145, 1302, 1356, 1535, 1647, 1719, 2864, 2928, 3308; Anal. calcd. for $C_{31}H_{48}{}^{35}ClN_5O_8$: C, 56.91; H, 7.40; N, 10.71%; Found: C, 57.06; H, 7.69; N, 10.45%.

*Cyclooctyne **15a** bearing a biotin moiety conjugated with a pentamethylene linker*

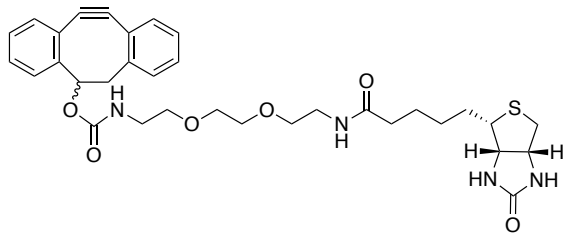


Colorless solid; Mp 112–115 °C; TLC R_f = 0.25 (CH_2Cl_2/MeOH = 10/1); HPLC analysis: the diastereomers did not separate and observed as a single peak with R*t* = 17.2 min [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; Cyclooctyne **15a** is a mixture of diastereomers and conformational isomers were observed by NMR analysis. For the major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19–1.64 (m, 13H), 2.08–2.17 (m, 2H), 2.63–2.67 (m, 1H), 2.83–2.91 (m, 2H), 3.08–3.18 (m, 5H), 4.21–4.30 (br, 1H), 4.39–4.46 (br, 1H), 5.47 (s, 1H), 7.25–7.37 (m, 7H), 7.48–7.50 (m, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ 25.2 (1C), 27.1 (1C), 29.6 (1C), 29.9 (1C), 30.1 (1C), 30.7 (1C), 36.9 (1C), 40.4 (1C), 41.2 (1C), 41.8 (1C), 47.3 (1C), 57.1 (1C), 61.7 (1C), 63.5 (1C), 78.0 (1C), 111.1 (1C), 114.0 (1C), 122.6 (1C), 125.07 (1C), 125.13 (1C), 127.1 (1C), 127.3 (1C), 128.4 (1C), 128.5 (1C), 129.4 (1C), 129.5 (1C), 131.2 (1C), 152.6 (1C), 153.9 (1C), 158.2 (1C), 166.2 (1C), 176.1 (1C); IR (KBr, cm^{-1}) 757, 1023, 1262, 1450, 1532, 1695, 1650, 2927, 3290; HRMS (ESI$^+$) *m*/*z* 575.2678 ([M+H]$^+$, $C_{32}H_{39}N_4O_4S^+$ requires 575.2687).

HPLC chart:

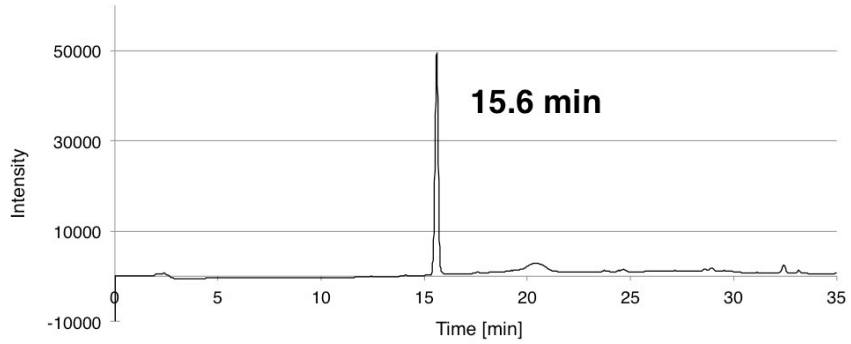


*Cyclooctyne **15b** bearing a biotin moiety conjugated with a 3,6-dioxaoctamethylene (PEO_2) linker*

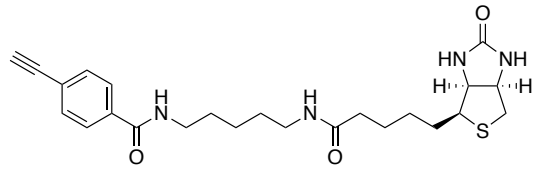


Colorless solid; Mp 80 °C (dec.); TLC R_f = 0.38 (CH_2Cl_2/MeOH = 10/1); HPLC analysis: the diastereomers did not separate and observed as a single peak with R*t* = 15.6 min [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; Cyclooctyne **15b** is a mixture of diastereomers and conformational isomers were observed by NMR analysis. For the major isomer: ^{1}H NMR (400 MHz, CD_3OD) δ 1.38–1.73 (m, 6H), 2.18 (t, 2H, *J* = 7.0 Hz), 2.66–2.69 (m, 1H), 2.81–2.90 (m, 2H), 3.14–3.80 (m, 14H), 4.22–4.24 (m, 1H), 4.43–4.45 (m, 1H), 5.42 (s, 1H), 7.32–7.58 (m, 8H); ^{13}C NMR (126 MHz, CD_3OD) δ 26.9 (1C), 29.6 (1C), 29.8 (1C), 36.8 (1C), 40.4 (1C), 41.1 (1C), 41.9 (1C), 47.2 (1C), 57.1 (1C), 61.7 (1C), 63.4 (1C), 70.7 (1C), 71.0 (1C), 71.4 (1C), 71.44 (1C+1C, two signals overlapped), 78.1 (1C), 111.1 (1C), 113.9 (1C), 122.5 (1C), 125.1 (1C), 127.0 (1C), 127.3 (1C), 128.4 (1C), 128.5 (1C), 129.4 (1C), 129.4 (1C), 131.2 (1C), 152.6 (1C), 153.8 (1C), 158.1 (1C), 166.2 (1C), 176.2 (1C); IR (KBr, cm^{-1}) 763, 1038, 1139, 1264, 1428, 1694, 2472, 2928, 3268; HRMS (ESI^+) m/z 643.2548 ($[M+Na]^+$, $C_{33}H_{40}N_4NaO_6S^+$ requires 643.2561).

HPLC chart:

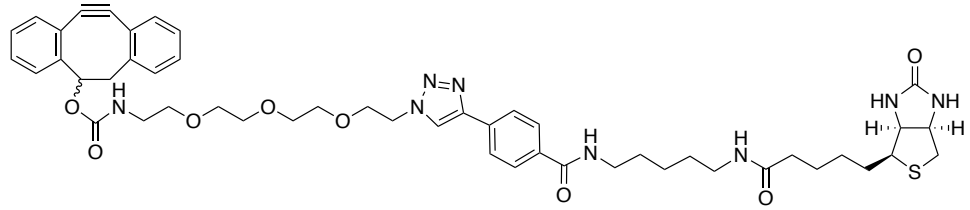


4-Ethynyl-*N*-(5-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)pentyl)benzamide (**S8**)



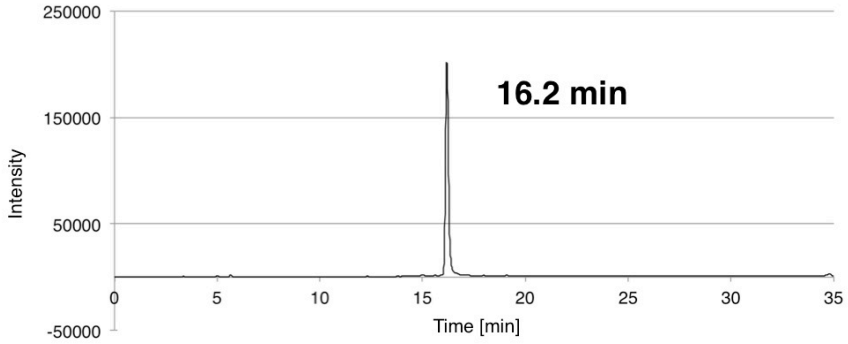
Colorless solid; Mp 170–172 °C; TLC R_f = 0.29 (CH_2Cl_2/MeOH = 10/1). ^{1}H NMR (400 MHz, CD_3OD) δ 1.40–1.44 (m, 4H), 1.54–1.78 (m, 8H), 2.18 (t, 2H, *J* = 7.6 Hz), 2.70 (d, 1H, *J* = 12.4 Hz), 2.92 (dd, 1H, *J* = 4.8, 12.4 Hz), 3.12–3.20 (m, 3H), 3.38 (t, 2H, *J* = 7.2 Hz), 3.67 (s, 1H), 4.28–4.31 (m, 1H), 4.47–4.50 (m, 1H), 7.53–7.58 (AA'BB', 2H), 7.78–7.82 (AA'BB', 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 25.4 (1C), 27.0 (1C), 29.6 (1C), 29.9 (1C), 30.2 (1C+1C, two signals overlapped), 36.9 (1C), 40.3 (1C), 41.0 (1C), 41.1 (1C), 57.1 (1C), 61.7 (1C), 63.5 (1C), 81.2 (1C), 83.7 (1C), 127.0 (1C), 128.5 (2C), 133.2 (2C), 135.9 (1C), 166.2 (1C), 169.4 (1C), 176.1 (1C); IR (KBr, cm^{-1}) 1323, 1465, 1546, 1638, 1696, 2930, 3290; HRMS (ESI^+) *m*/*z* 457.2288 ($[M+H]^+$, $C_{24}H_{33}N_4O_3S^+$ requires 457.2268).

*Cyclooctyne **15c** bearing a biotin moiety conjugated with 3,6,9-trioxaundecamethylene (PEO_3) and pentamethylene linkers linked by a 1,2,3-triazole skeleton*

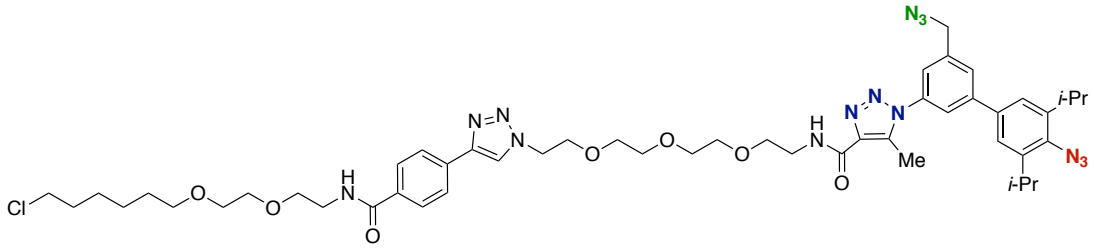


Colorless solid; Mp 131 °C (dec.); TLC R_f = 0.48 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: the diastereomers did not separate and observed as a single peak with R*t* = 16.2 min [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; Cyclooctyne **15c** is a mixture of diastereomers and conformational isomers. For the major isomer: ^{1}H NMR (400 MHz, CD_3OD) δ 1.37–1.68 (m, 12H), 2.15–2.18 (m, 2H), 2.66–2.88 (m, 3H), 3.16–3.62 (m, 18H), 3.90–3.92 (m, 2H), 4.24–4.27 (m, 1H), 4.43–4.47 (m, 1H), 4.60–4.62 (m, 2H), 5.39–5.43 (m, 1H), 7.26–7.37 (m, 7H), 7.52–7.53 (m, 1H), 7.84–7.95 (m, 4H), 8.43 (s, 1H); IR (KBr, cm^{-1}) 768, 1105, 1258, 1450, 1635, 1696, 2475, 2928; HRMS (ESI^+) *m*/*z* 943.4130 ($[M+Na]^+$, $C_{49}H_{60}N_8NaO_8S^+$ requires 943.4147).

HPLC chart:

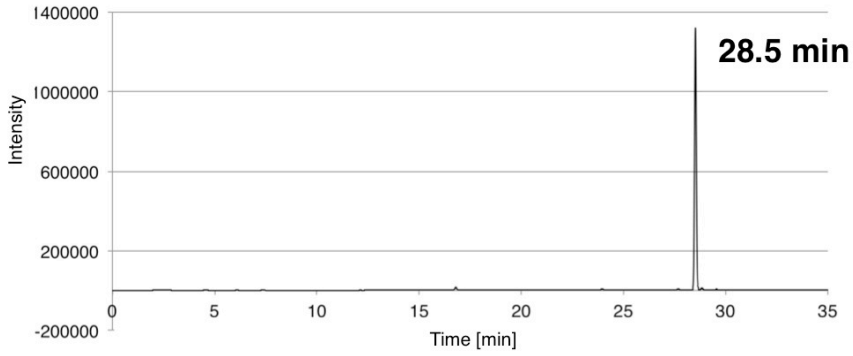


1-(4′-Azido-5-azidomethyl-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)-*N*-(2-(2-(2-(2-(4-(4-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)carbamoyl)phenyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide (**S9**)

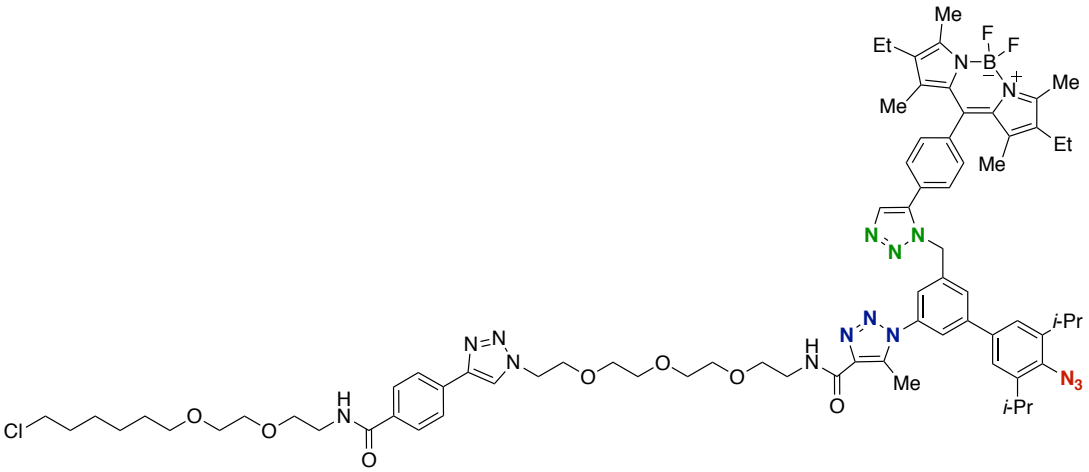


Pale orange oil; TLC R_f = 0.62 (CH_2Cl_2/MeOH = 10/1); HPLC analysis: R*t* = 28.5 min [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.45 (m, 16H), 1.53–1.62 (m, 2H), 1.69–1.77 (m, 2H), 2.64 (s, 3H), 3.44–3.50 (m, 6H), 3.59–3,69 (m, 20H), 3.95 (t, 2H, *J* = 4.8 Hz), 4.56 (s, 2H), 4.62 (t, 2H, *J* = 4.8 Hz), 6.82 (s, 1H), 7.32 (s, 2H), 7.37 (s, 1H), 7.57–7.64 (m, 3H), 7.84 (d, 2H, *J* = 8.4 Hz), 7.89 (d, 2H, *J* = 8.4 Hz), 8.10 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 9.8 (1C), 23.5 (4C), 25.4 (1C), 26.6 (1C), 28.9 (2C), 29.4 (1C), 32.5 (1C), 38.7 (1C), 39.7 (1C), 45.0 (1C), 50.4 (1C), 54.0 (1C), 69.4 (1C), 69.7 (1C), 69.8 (1C), 70.0 (1C), 70.2 (1C), 70.4 (1C), 70.53 (1C), 70.55 (1C), 70.61 (1C), 71.3 (1C), 121.7 (1C), 123.0 (2C), 123.2 (1C), 123.7 (1C), 125.5 (2C), 127.6 (2C), 128.0 (1C), 133.77 (1C), 133.78 (1C), 135.7 (1C), 136.4 (1C), 136.8 (1C), 137.3 (1C), 138.1 (1C), 138.6 (1C), 143.7 (1C), 144.0 (2C), 146.6 (1C), 161.2 (1C), 167.0 (1C); IR (KBr, cm^{-1}) 862, 1111, 1287, 1460, 1518, 1659, 2102, 2932; HRMS (ESI^+) *m*/*z* 1033.4853 ($[M+Na]^+$, $C_{50}H_{67}{}^{35}ClN_{14}NaO_7^+$ requires 1033.4898).

HPLC chart:

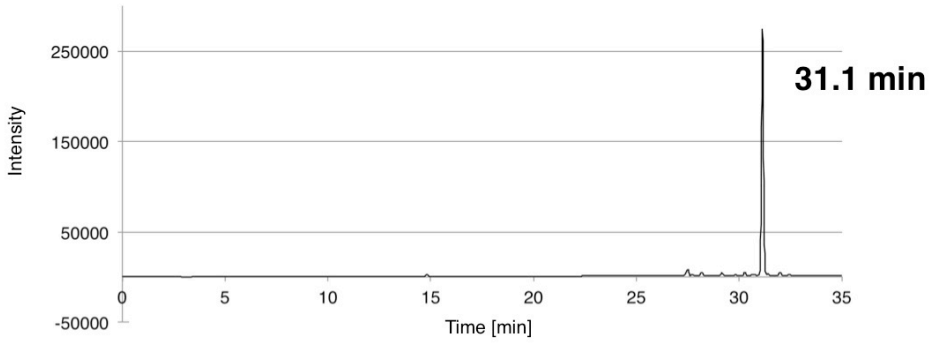


10-(4-(1-((4′-Azido-5-(4-((2-(2-(2-(2-(4-(4-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)carbamoyl)phenyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)carbamoyl)-5-methyl-1*H*-1,2,3-triazol-1-yl)-3′,5′-diisopropyl-1,1′-biphenyl-3-yl)methyl)-1*H*-1,2,3-triazol-5-yl)phenyl)-2,8-diethyl-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*][1,3,2]diazaborinin-4-ium-5-uide (**S10**)

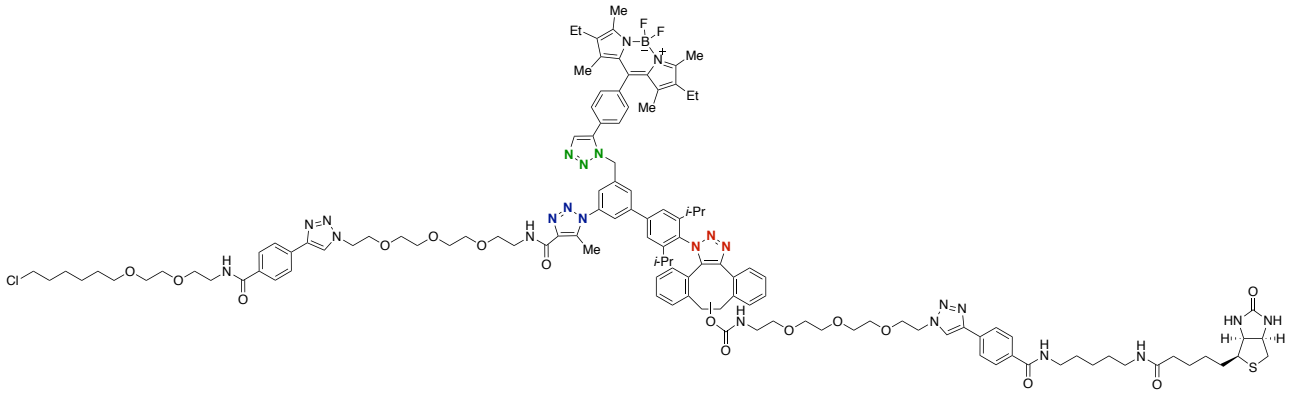


Pink solid; Mp 62 °C (dec.); TLC R_f = 0.41 (CH_2Cl_2/MeOH = 10/1); HPLC analysis: R*t* = 31.1 min [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, 6H, *J* = 7.2 Hz), 1.17–1.45 (m, 22H), 1.54–1.63 (m, 2H), 1.68–1.77 (m, 2H), 2.23 (q, 4H, *J* = 7.6 Hz), 2.52 (s, 6H), 2.56 (s, 3H), 3.37–3.50 (m, 6H), 3.58–3.68 (m, 20H), 3.92 (t, 2H, *J* = 4.8 Hz), 4.59 (t, 2H, *J* = 4.8 Hz), 5.80 (s, 2H), 6.82 (br s, 1H), 7.12 (s, 1H), 7.24 (s, 2H), 7.40–7.43 (m, 3H), 7.50–7.54 (m, 4H), 7.84–7.90 (m, 5H), 8.09 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 9.7 (1C), 11.7 (2C), 12.5 (1C), 14.5 (2C), 17.0 (2C), 23.4 (4C), 25.4 (1C), 26.6 (2C), 28.9 (2C), 29.4 (1C), 29.7 (1C), 32.4 (1C) 38.7 (1C), 39.7 (1C), 45.0 (1C), 50.4 (1C), 51.3 (1C), 69.4 (1C), 69.7 (1C), 69.8 (1C), 70.0 (1C), 70.2 (1C), 70.4 (1C), 70.5 (1C), 70.6 (1C), 71.3 (1C), 121.7 (1C), 122.2 (1C), 122.8 (2C), 123.9 (1C), 125.5 (2C), 126.8 (2C), 127.1 (2C), 127.6 (2C), 129.2 (2C), 129.5 (2C), 130.4 (1C), 133.1 (1C), 133.75 (2C), 133.79 (1C+1C, two signals overlapped), 135.9 (1C), 136.6 (1C), 136.7 (1C), 136.9 (1C), 137.5 (1C), 137.6 (2C+1C, two signals overlapped), 137.7 (1C), 138.0 (1C), 138.6 (1C), 143.9 (1C), 144.1 (2C), 146.6 (1C), 154.4 (1C), 161.0 (1C), 167.0 (1C); FL (MeOH) λ_{max} = 538 nm; IR (KBr, cm^{-1}) 980, 1145, 1194, 1321, 1476, 1541, 1655, 2122, 2926; HRMS (ESI$^+$) *m*/*z* 1437.7143 ([M+Na]$^+$, $C_{75}H_{94}{}^{11}B^{35}ClF_2N_{16}NaO_7^+$ requires 1437.7133).

HPLC chart:

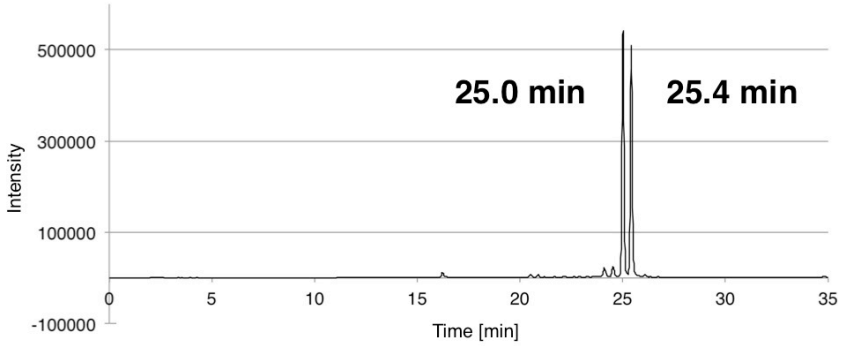


Trifucntional probe **16d**

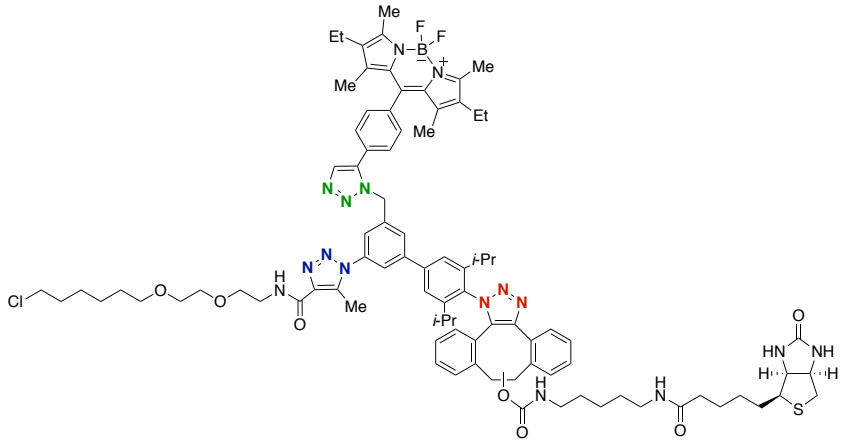


Pink solid; Mp 104 °C (dec.); TLC R_f = 0.38 (CH_2Cl_2/MeOH = 10/1); HPLC analysis: the diastereomers did not separate and two peaks for regioisomers were observed at R*t* = 25.0 min (51%) and 25.4 min (49%) [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; FL (MeOH) λ_{max} = 538 nm; IR (KBr, cm^{-1}) 979, 1193, 1471, 1544, 1652, 1703, 2928, 3303; HRMS (ESI^+) *m*/*z* 2359.1422 ($[M+Na]^+$, $C_{124}H_{154}{}^{11}B^{35}ClF_2N_{24}NaO_{15}S^+$ requires 2359.1494).

HPLC chart:

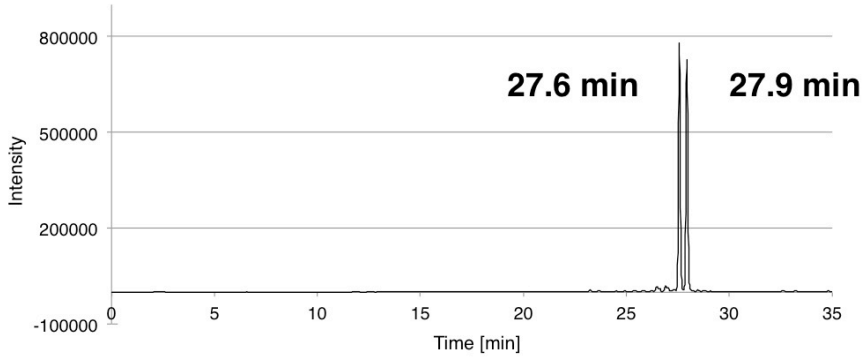


Trifucntional probe **16a**

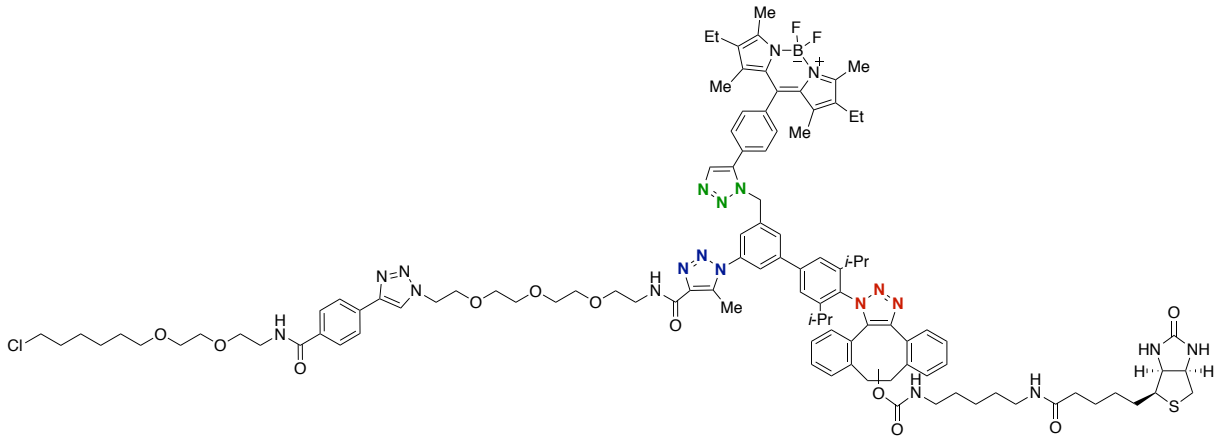


Pink solid; Mp 110 ℃ (dec.); TLC R_f = 0.10 (CH_2Cl_2/MeOH = 20/1); HPLC analysis: the diastereomers did not separate and two peaks for regioisomers were observed at R*t* = 27.6 min (51%) and 27.9 min (49%) [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; FL (MeOH) λ_{max} = 538 nm; IR (KBr, cm^{-1}) 737, 980, 1192, 1265, 1477, 1543, 1659, 1704, 2928, 3292; HRMS (ESI^+) *m*/*z* 1665.8106 ($[M+Na]^+$, $C_{90}H_{110}{}^{11}B^{35}ClF_2N_{16}NaO_7S^+$ requires 1665.8106).

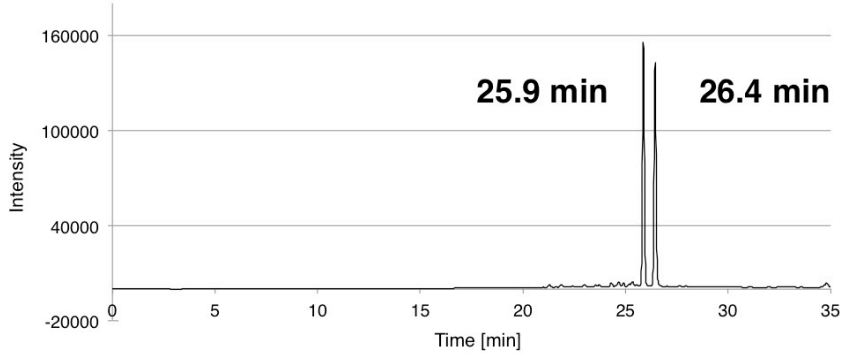
HPLC chart:



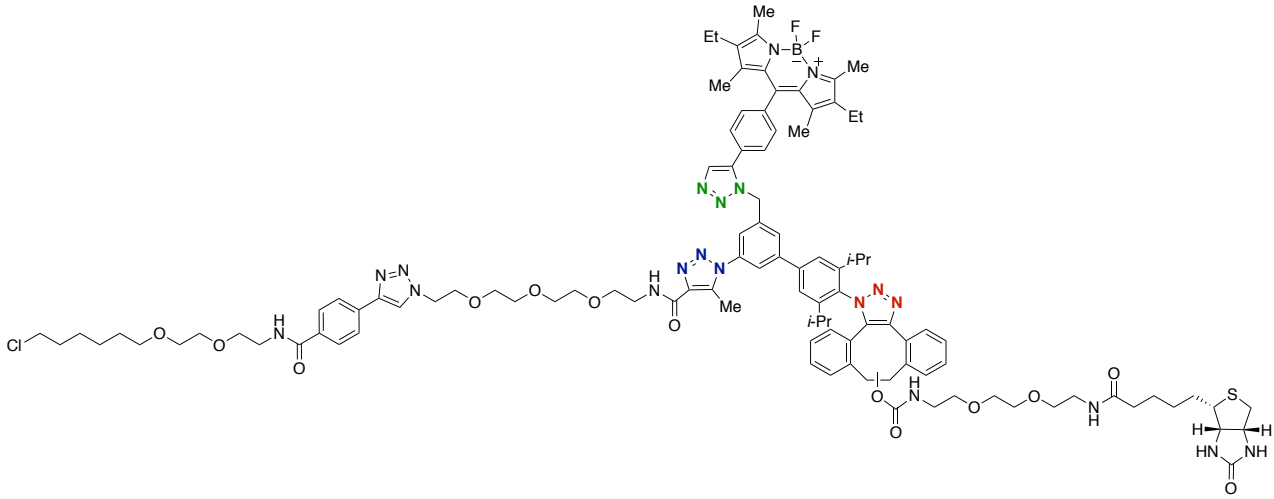
Trifucntional probe **16b**



Pink solid; Mp 122 °C (dec.); TLC R_f = 0.21 (CH_2Cl_2/MeOH = 20/1); HPLC analysis: the diastereomers did not separate and two peaks for regioisomers were observed at R*t* = 25.9 min (51%) and 26.4 min (49%) [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; FL (MeOH) λ_{max} = 538 nm; IR (KBr, cm^{-1}) 764, 979, 1117, 1193, 1543, 1694, 1703, 2928; HRMS (ESI^+) *m*/*z* 2011.9700 ($[M+Na]^+$, $C_{107}H_{132}{}^{11}B^{35}ClF_2N_{20}NaO_{11}S^+$ requires 2011.9747).

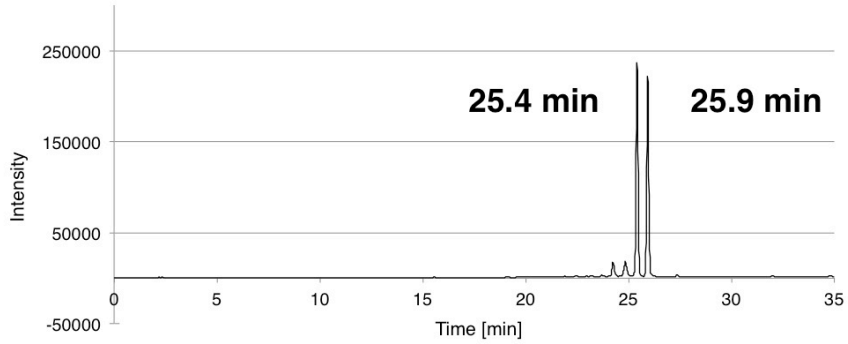


Trifucntional probe **16c**

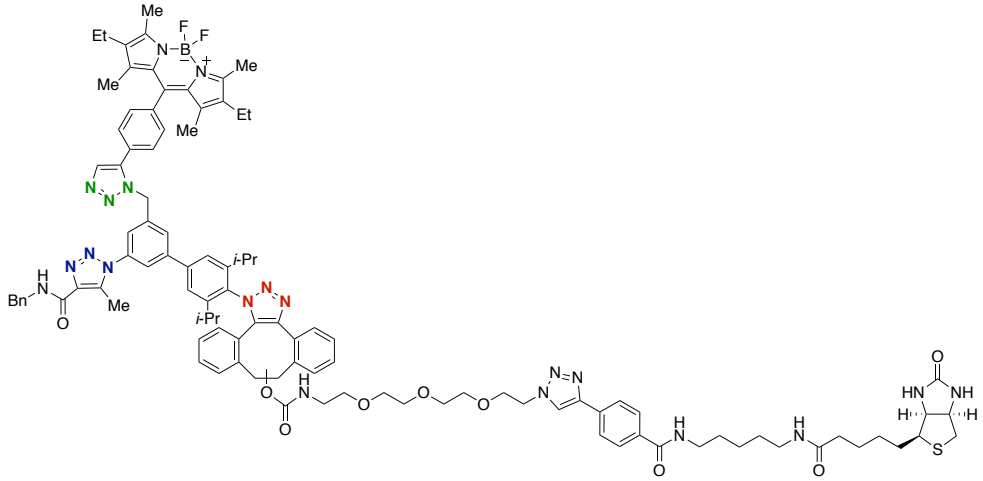


Pink solid; Mp 108 °C (dec.); TLC R_f = 0.24 (CH_2Cl_2/MeOH = 10/1); HPLC analysis: the diastereomers did not separate and two peaks for regioisomers were observed at R*t* = 25.4 min (52%) and 25.9 min (48%) [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; FL (MeOH) λ_{max} = 538 nm; IR (KBr, cm^{-1}) 742, 1076, 1084, 1117, 1195, 1460, 1543, 1649, 1693, 1693, 2929; HRMS (ESI^+) *m*/*z* 2058.9778 ($[M+Na]^+$, $C_{108}H_{134}{}^{11}B^{35}ClF_2N_{20}NaO_{13}S^+$ requires 2058.9835).

HPLC chart:

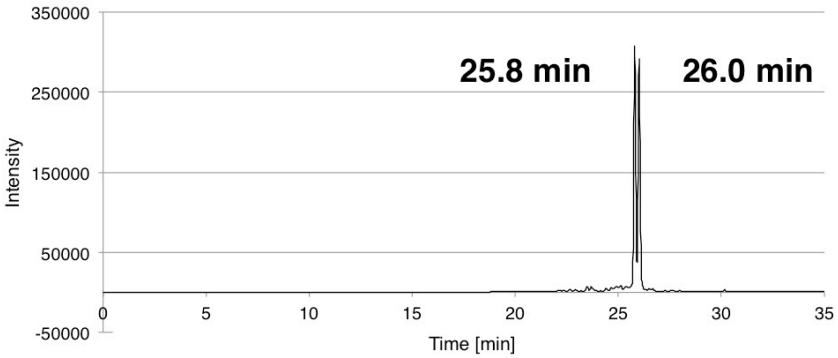


Bisfunctional tristriazole **S11**

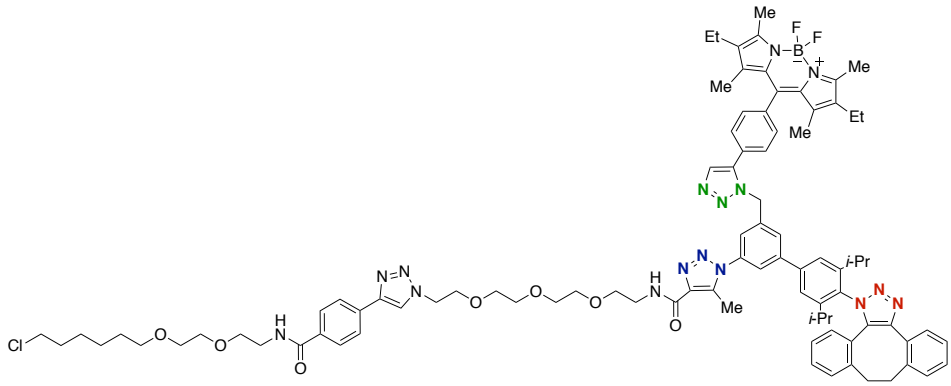


Pink solid; TLC R_f = 0.51 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: the diastereomers did not separate and two peaks for regioisomers were observed at R*t* = 25.8 min (51%) and 26.0 min (49%) [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; FL (MeOH) λ_{max} = 538 nm; HRMS (ESI$^+$) *m/z* 1895.9143 ([M+Na]$^+$, $C_{104}H_{119}{}^{11}BF_2N_{20}NaO_9S^+$ requires 1895.9143).

HPLC chart:

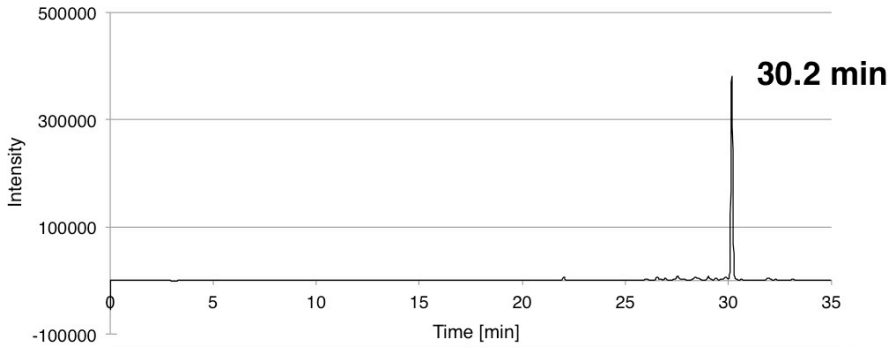


Bisfunctional tristriazole **S12**

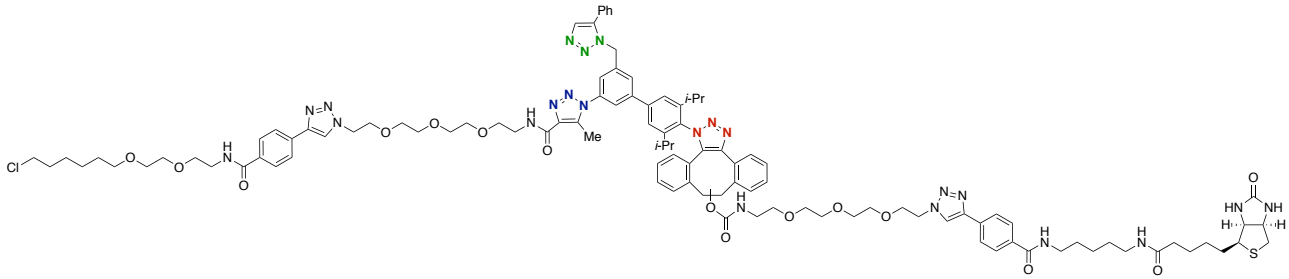


Pink solid; TLC R_f = 0.41 (CH_2Cl_2/MeOH = 10/1); HPLC analysis: Rt = 30.2 min [column: [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; FL (MeOH) λ_{max} = 538 nm; HRMS (ESI^+) m/z 1641.8057 ($[M+Na]^+$, $C_{91}H_{106}{}^{11}B^{35}ClF_2N_{16}NaO_7^+$ requires 1641.8072).

HPLC chart:

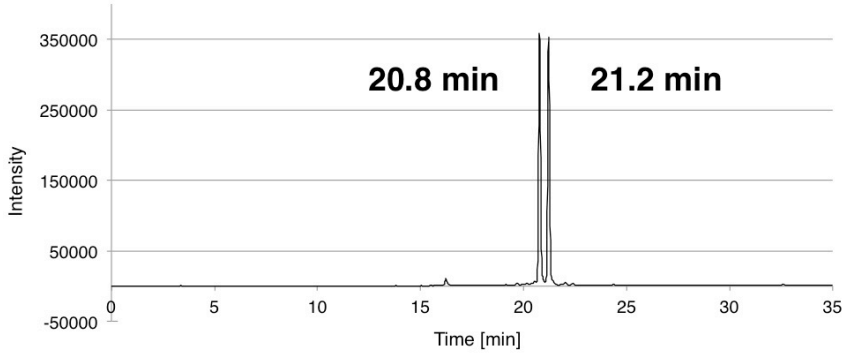


Bisfunctional tristriazole **S13**



Colorless solid; TLC R_f = 0.58 (CH_2Cl_2/MeOH = 6/1); HPLC analysis: the diastereomers did not separate and two peaks for regioisomers were observed at R*t* = 20.8 min (50%) and 21.2 min (50%) [column: Shiseido CAPCELL PAK MG II (4.6 mm i.d. × 250 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–25 min), 99:1 (25–35 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]; HRMS (ESI^+) *m*/*z* 2055.9575 ($[M+Na]^+$, $C_{107}H_{133}{}^{35}ClN_{22}NaO_{15}S^+$ requires 2055.9622).

HPLC chart:



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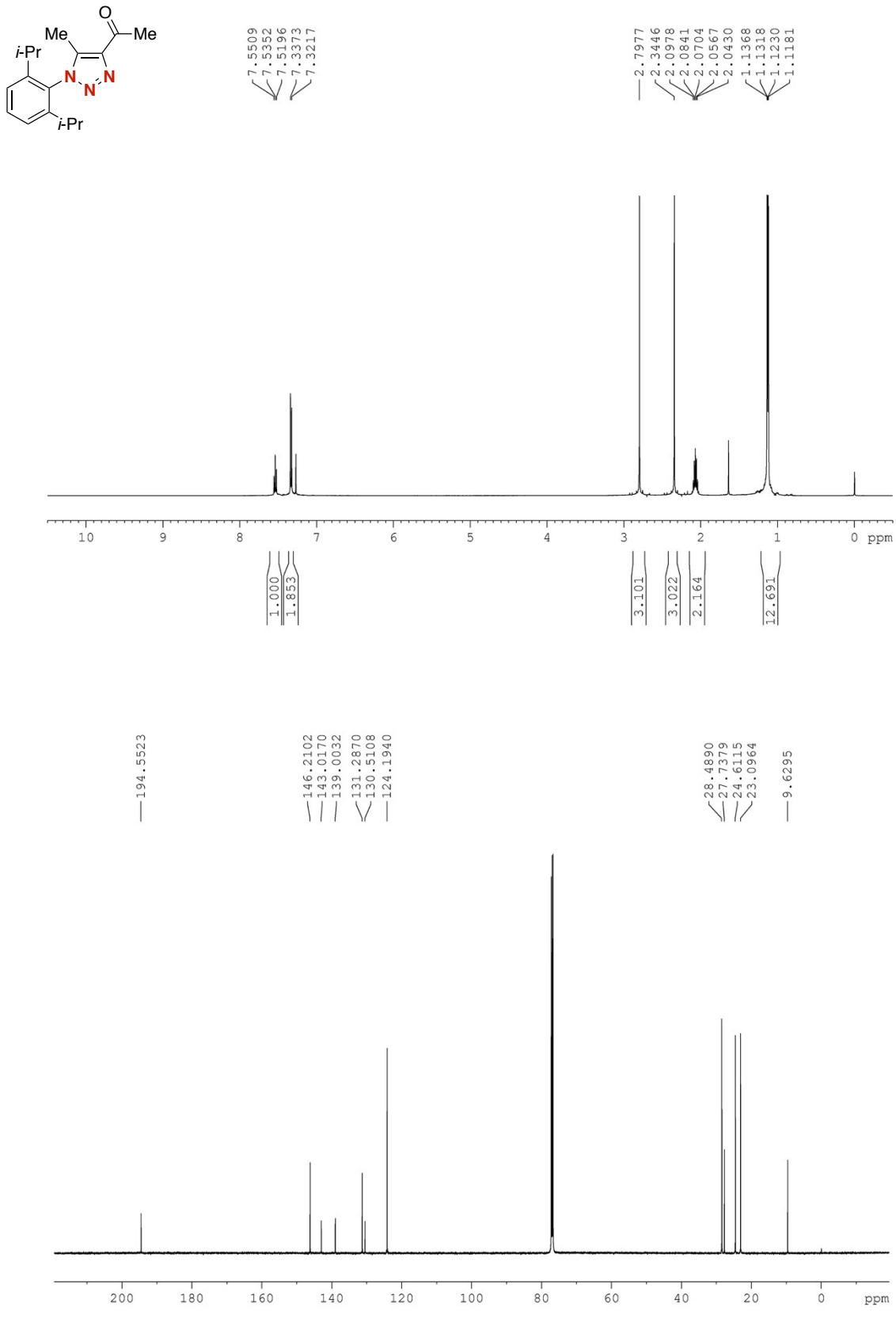
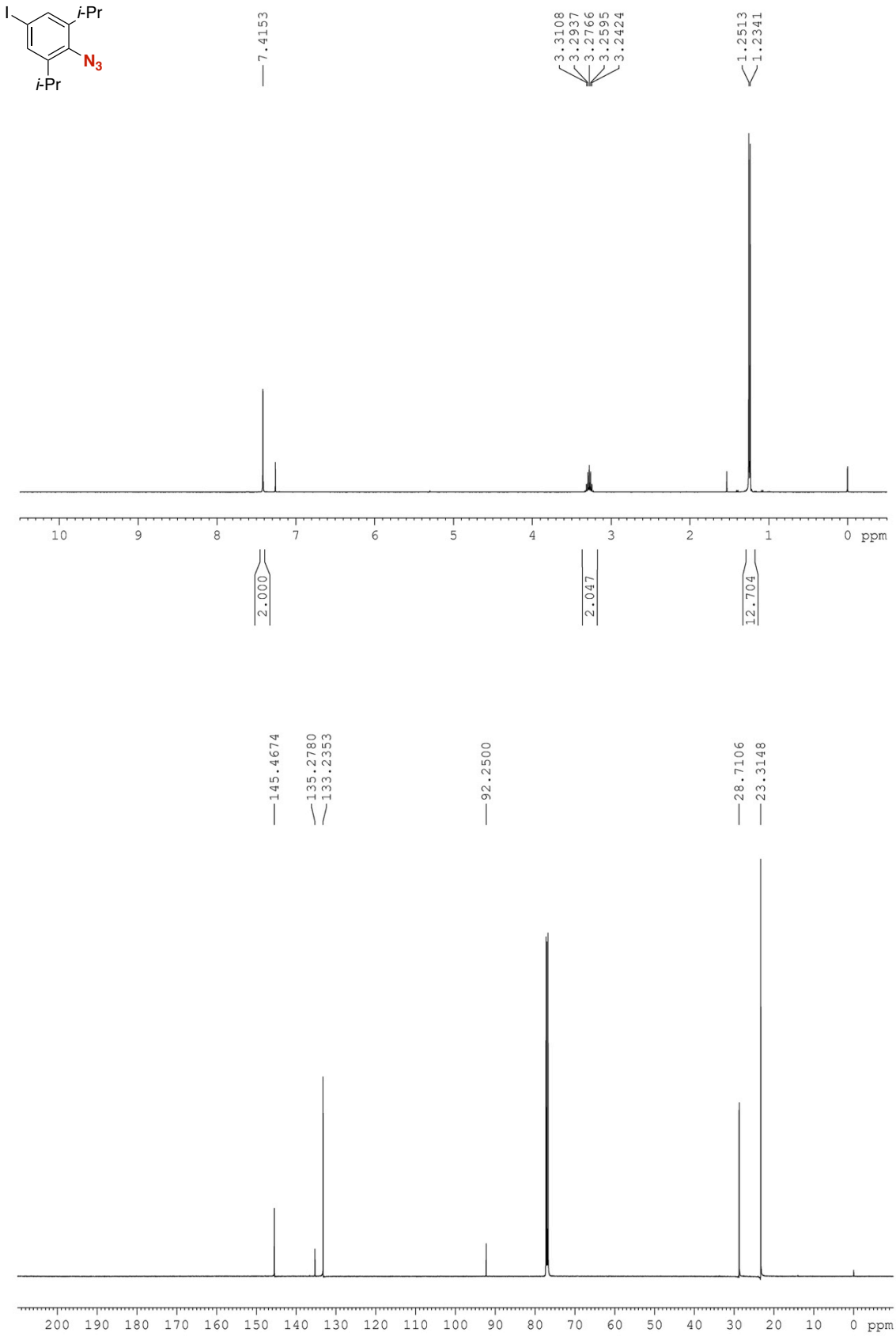
^{1}H and ^{13}C NMR Spectra of Compounds

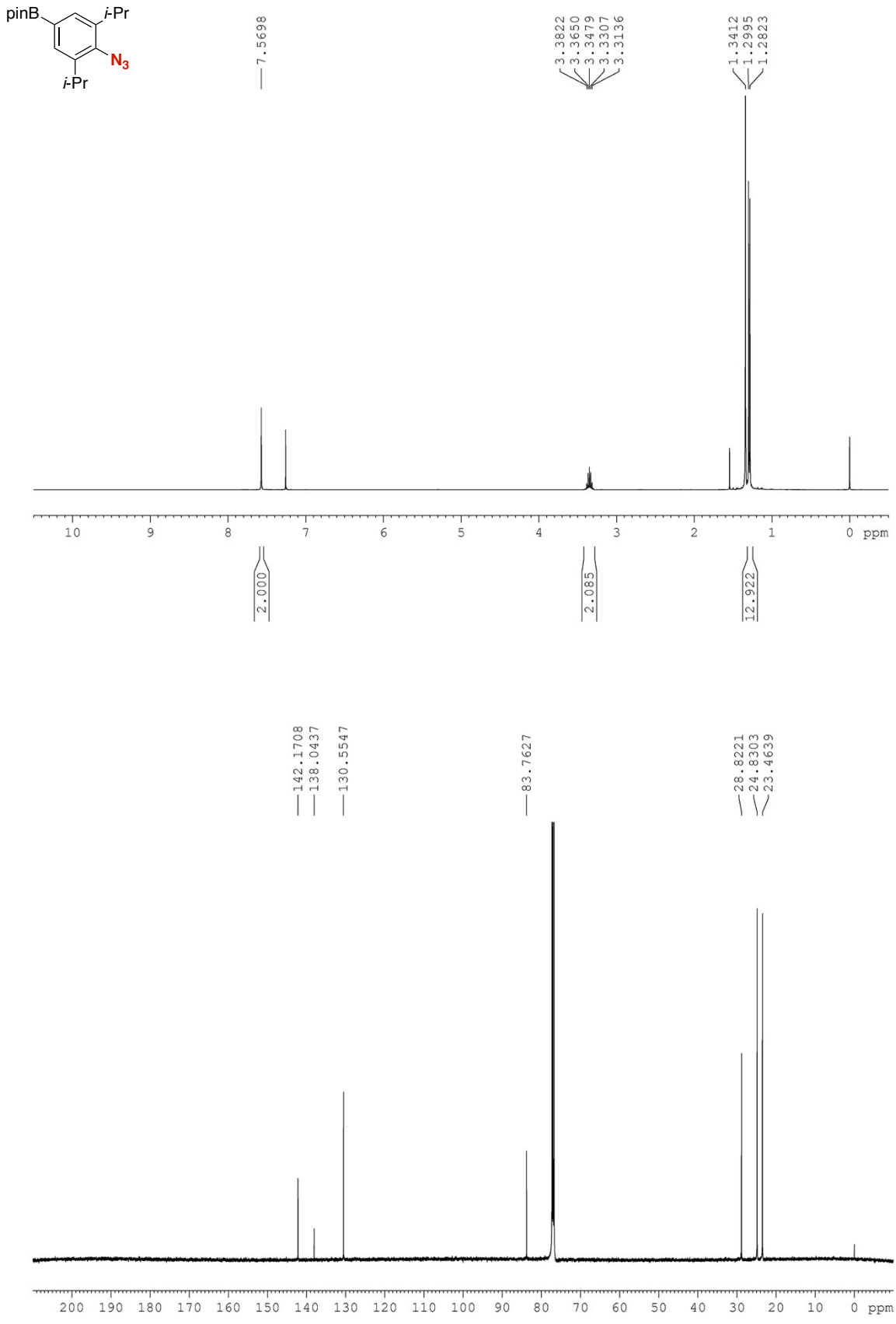
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **8a** ($CDCl_3$)



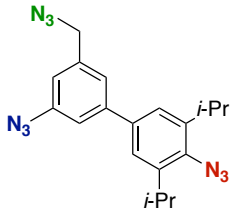
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S1** ($CDCl_3$)

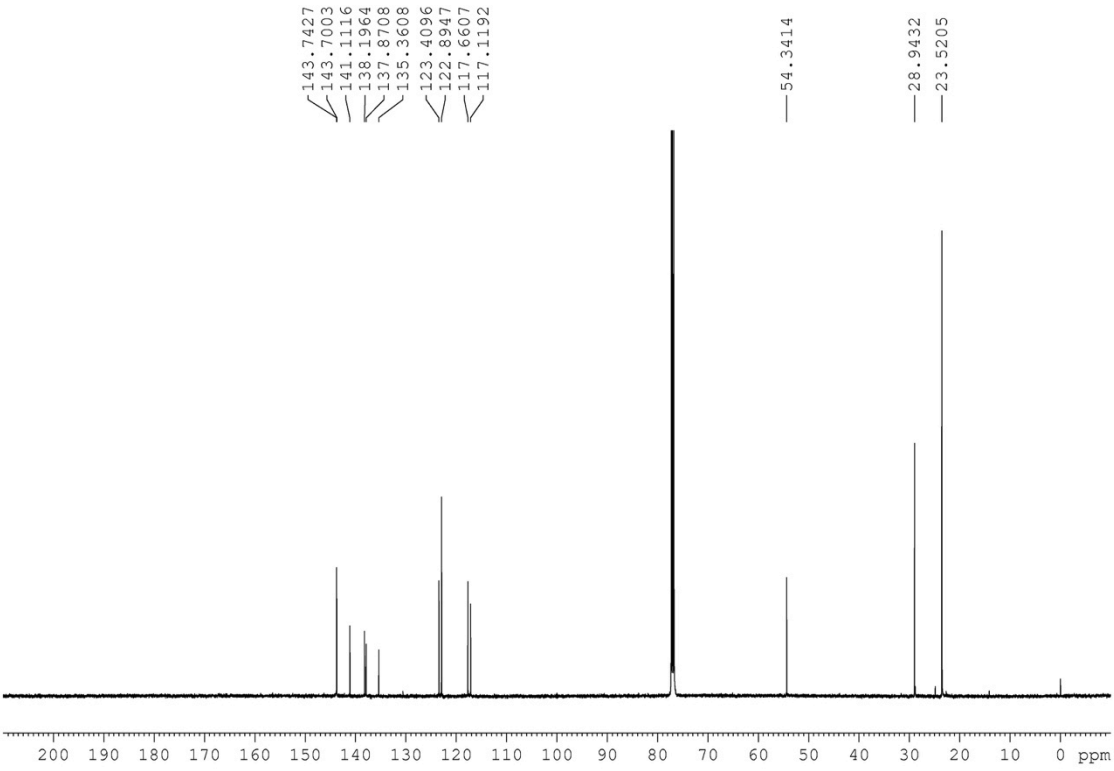


^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **10** ($CDCl_3$)

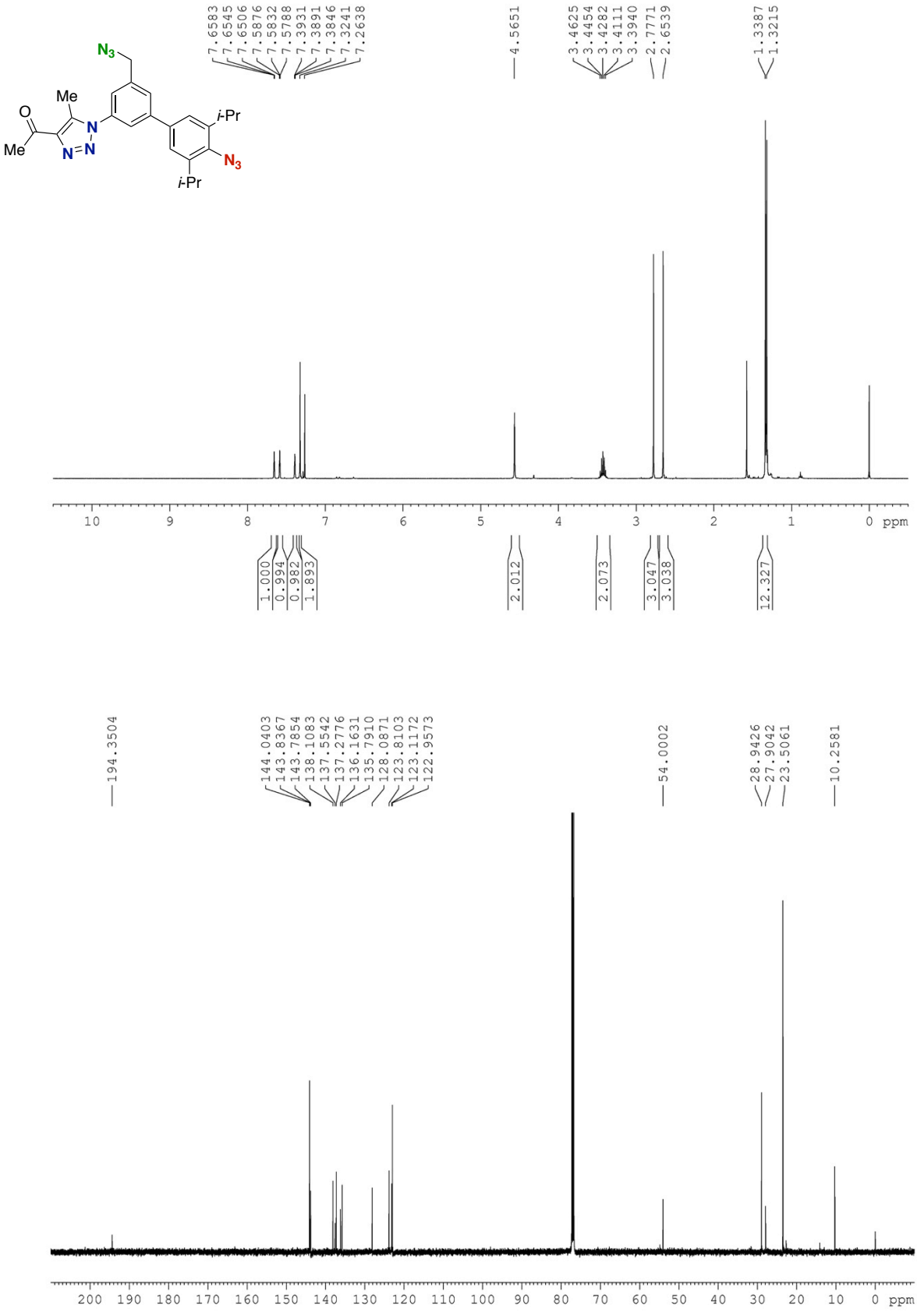


^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **11** ($CDCl_3$)

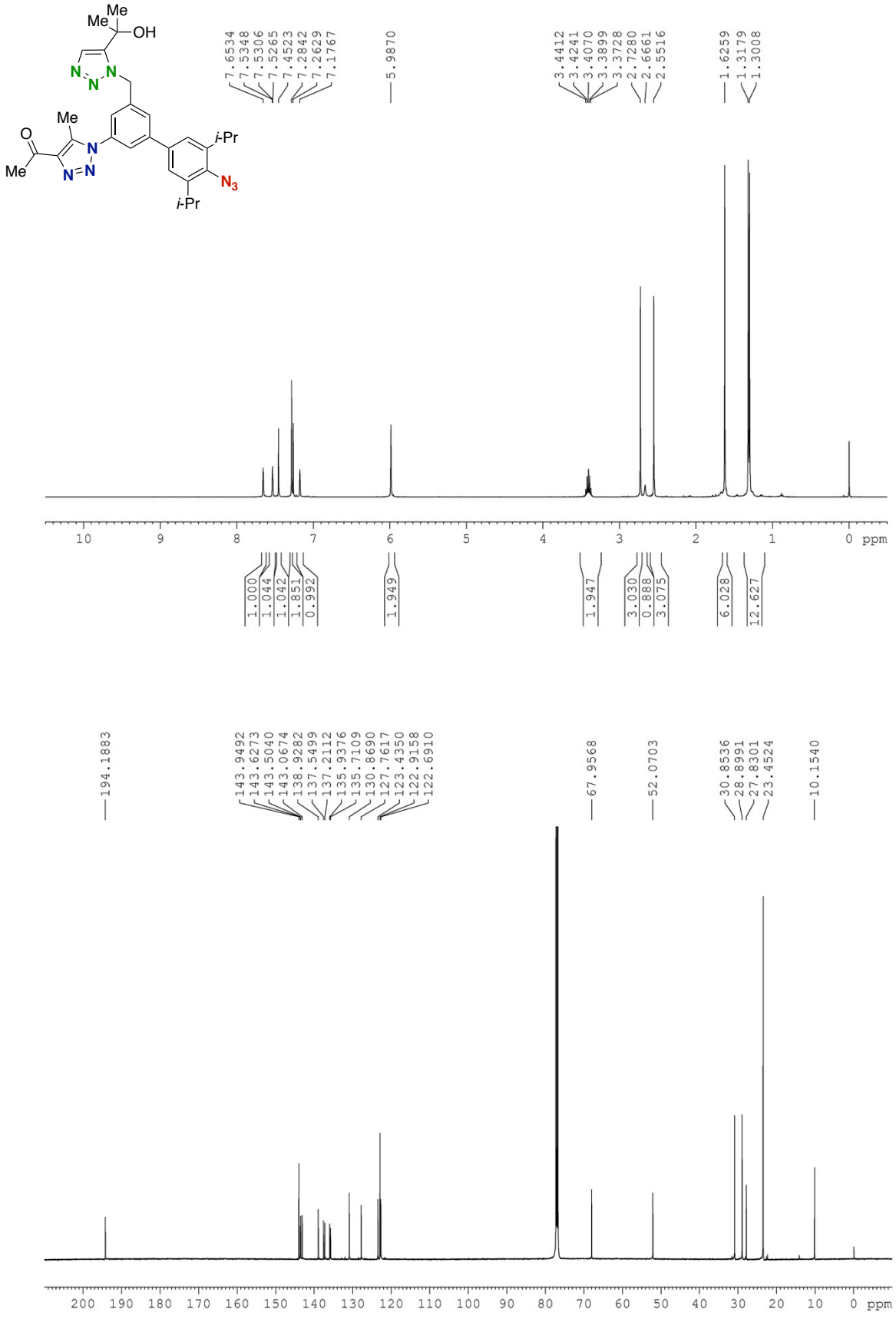




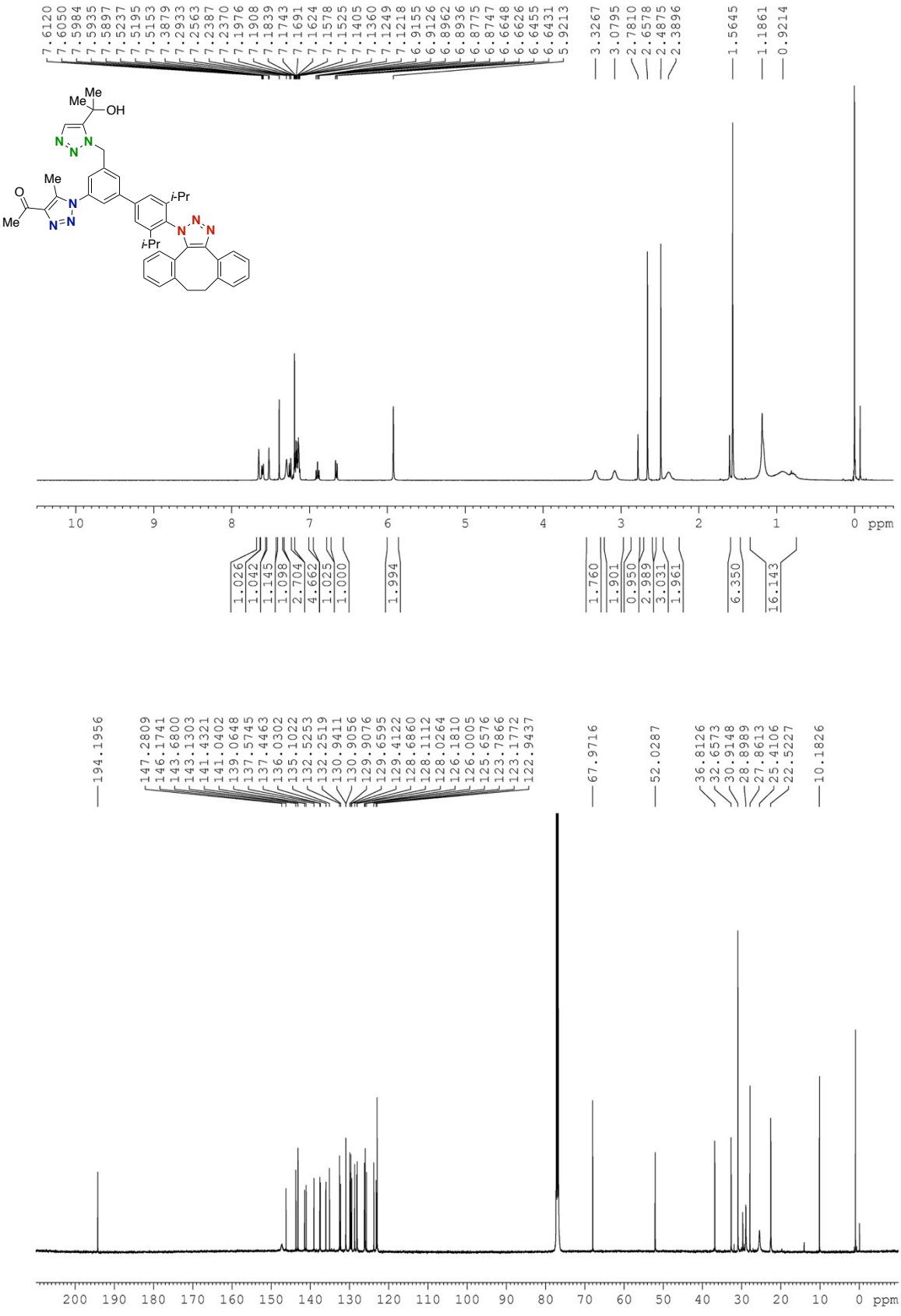
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S2** ($CDCl_3$)



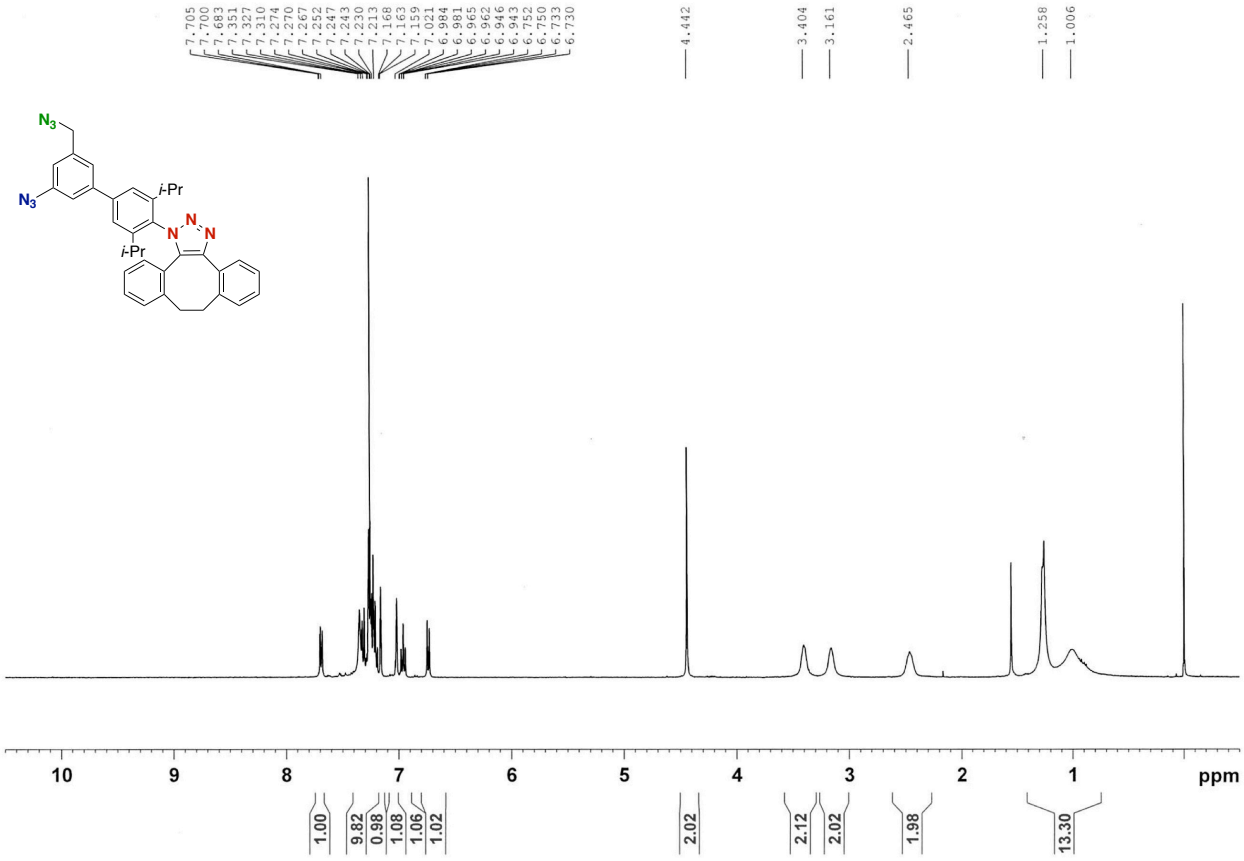
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S3** ($CDCl_3$)

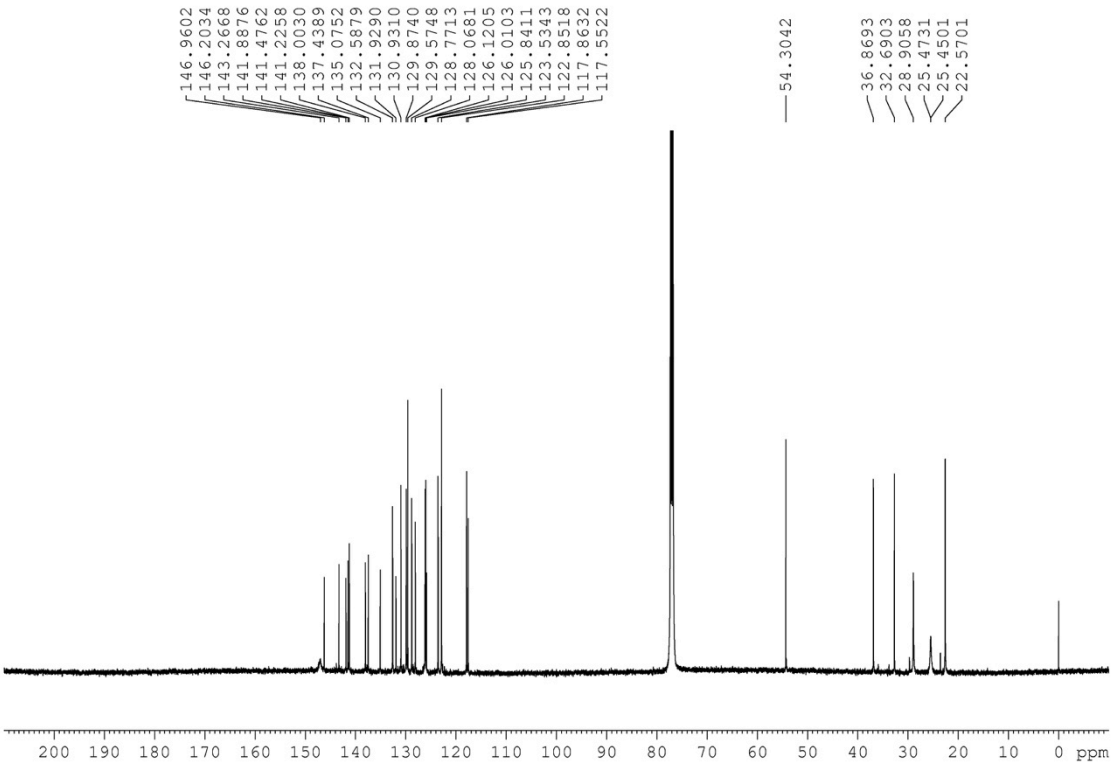


^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **12a** ($CDCl_3$)

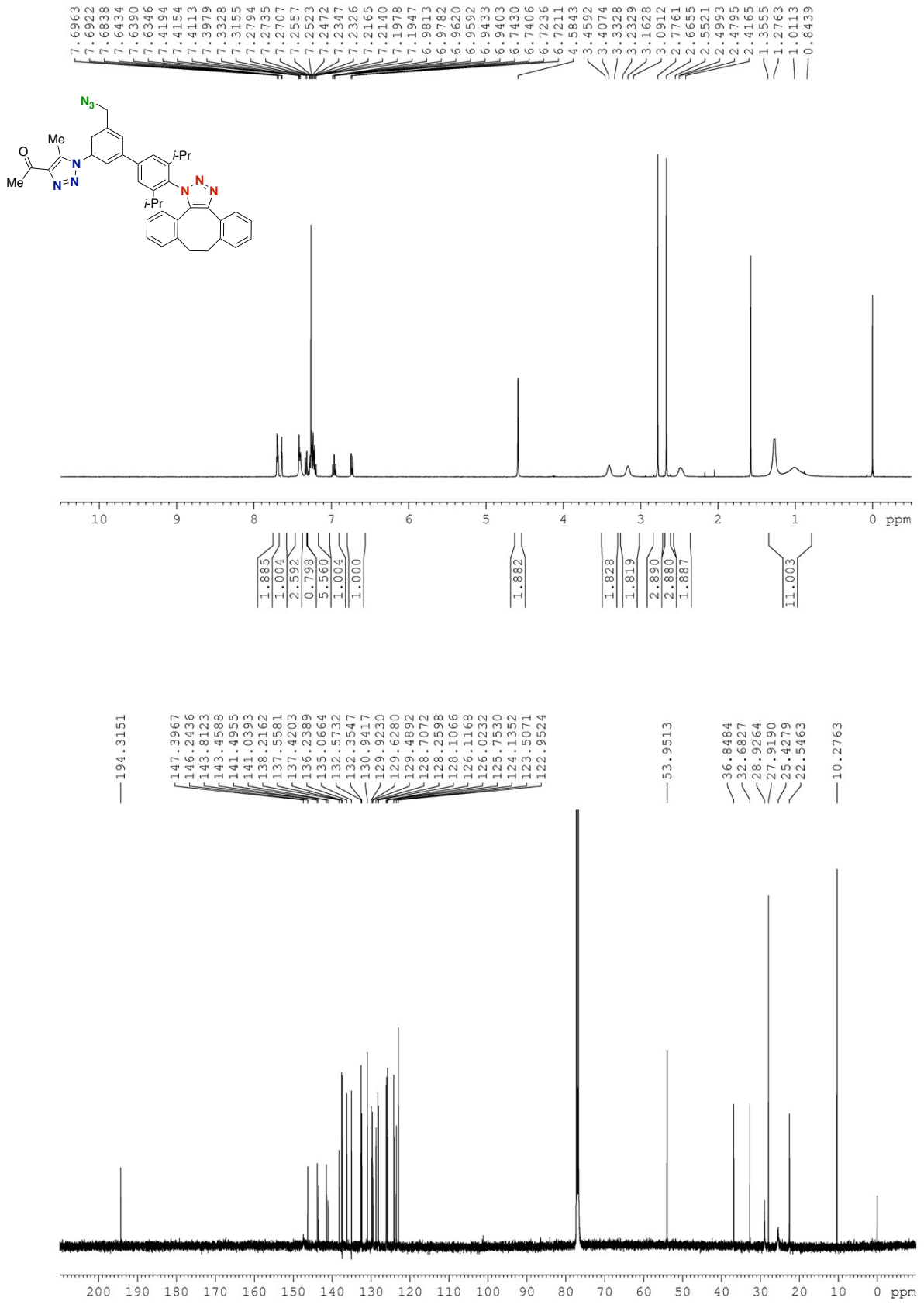


^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S4** ($CDCl_3$)

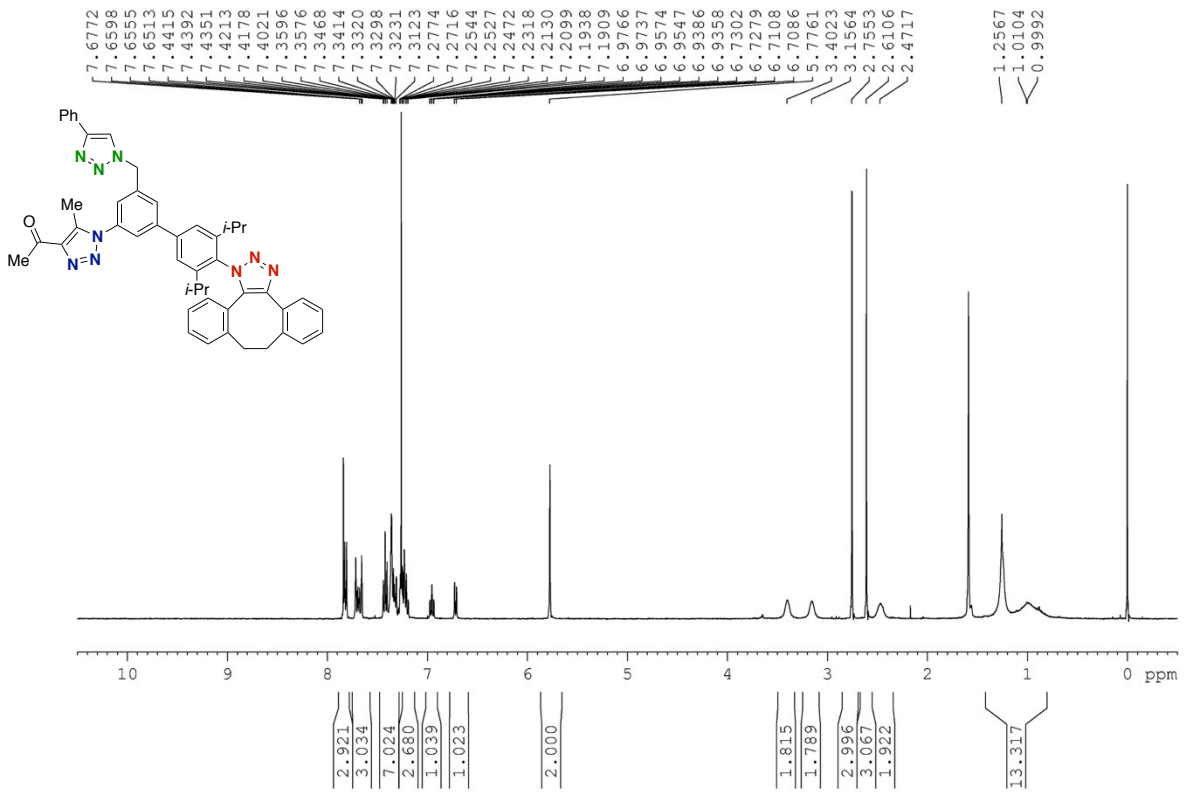


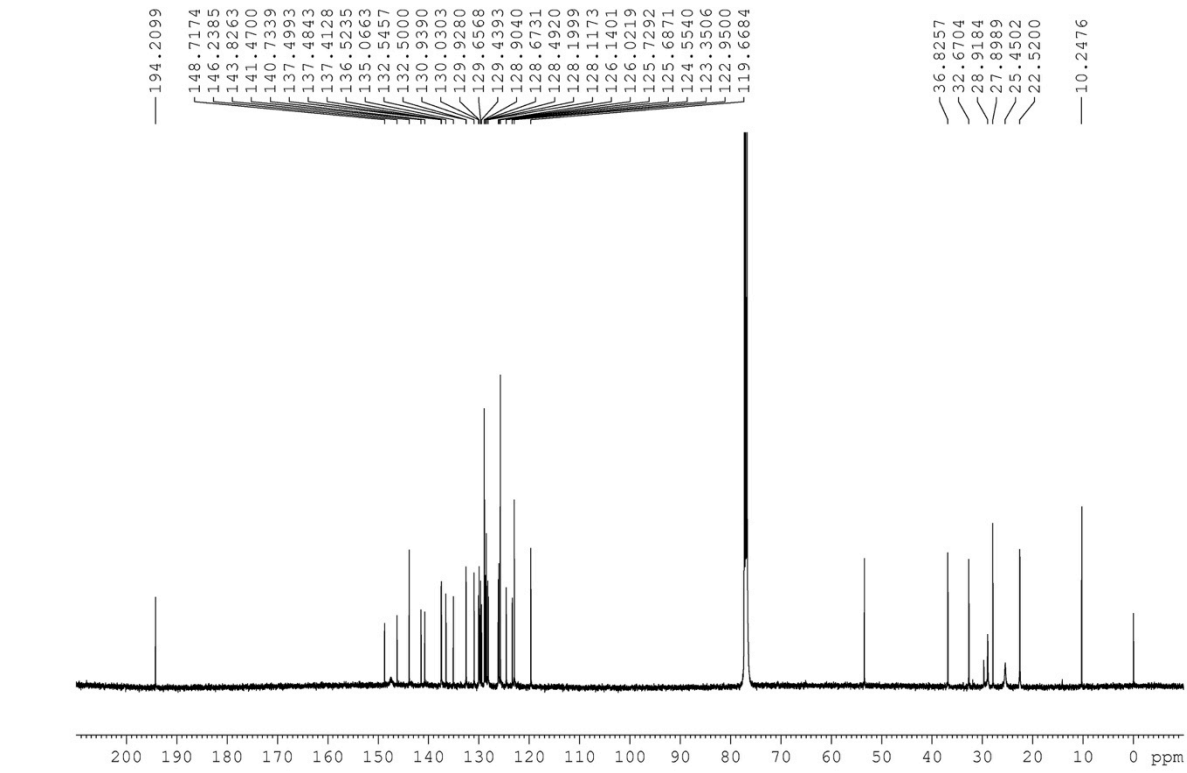


^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S5** ($CDCl_3$)

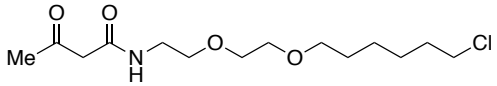


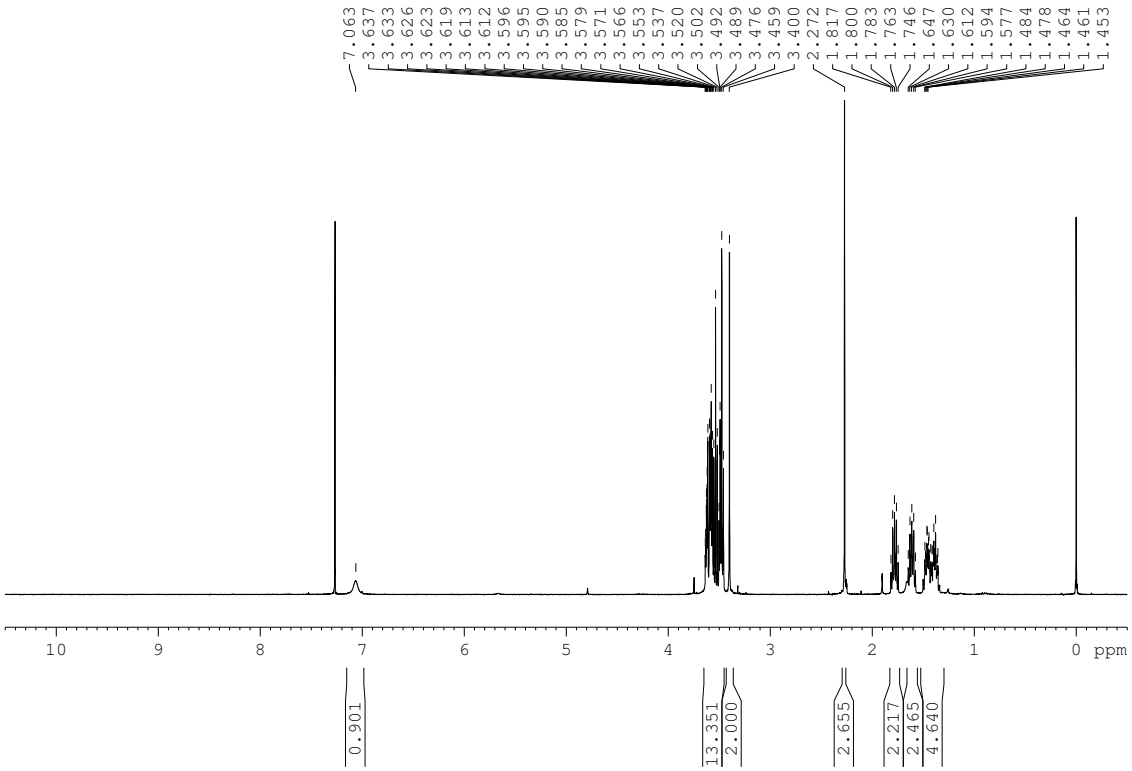
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **12b** ($CDCl_3$)

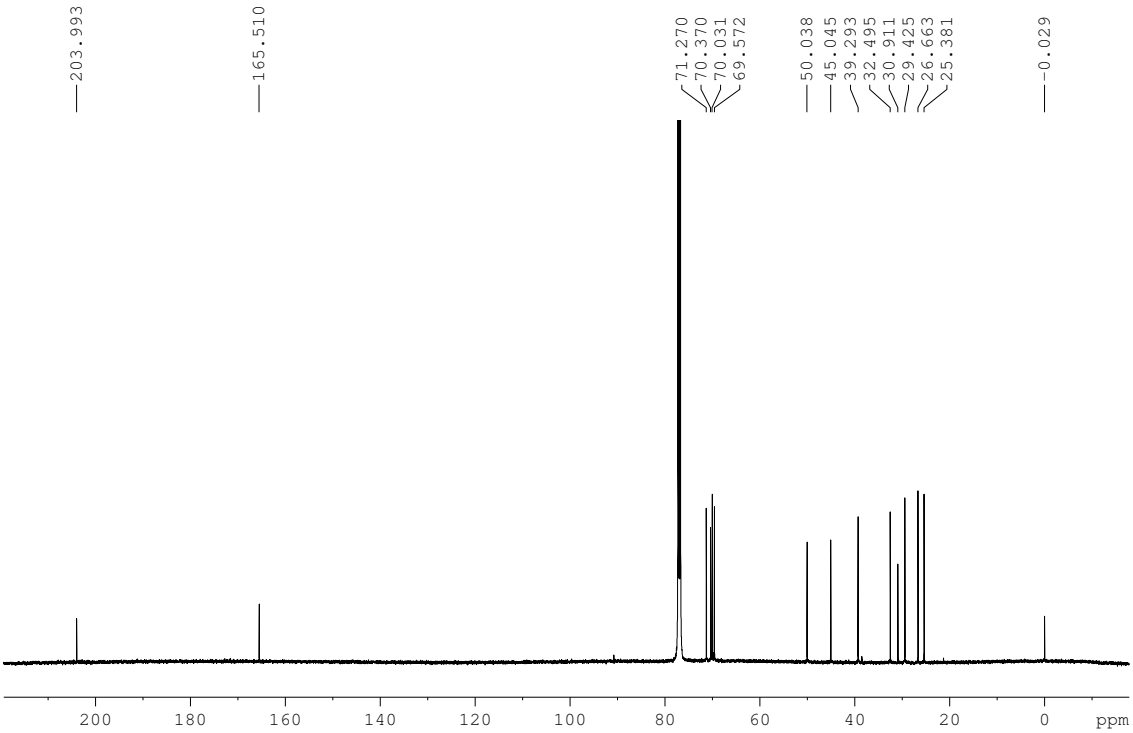




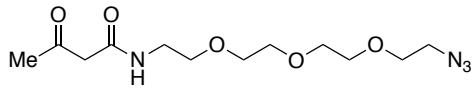
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **13a** ($CDCl_3$)

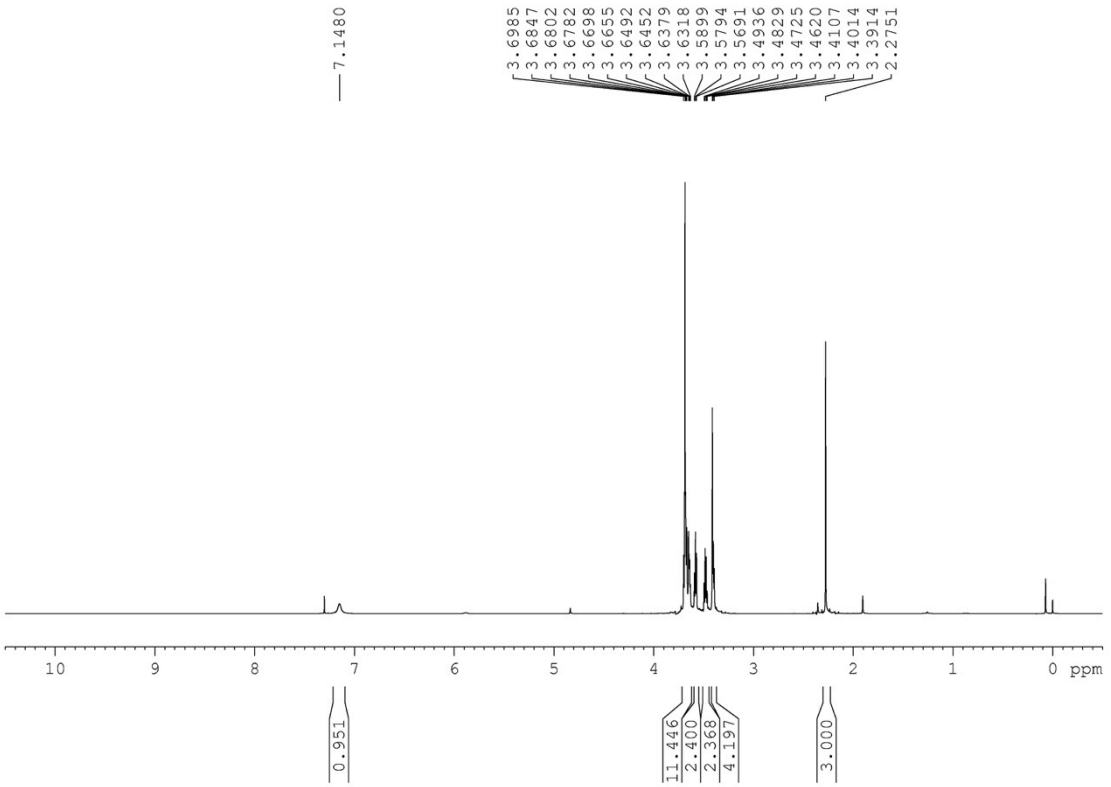


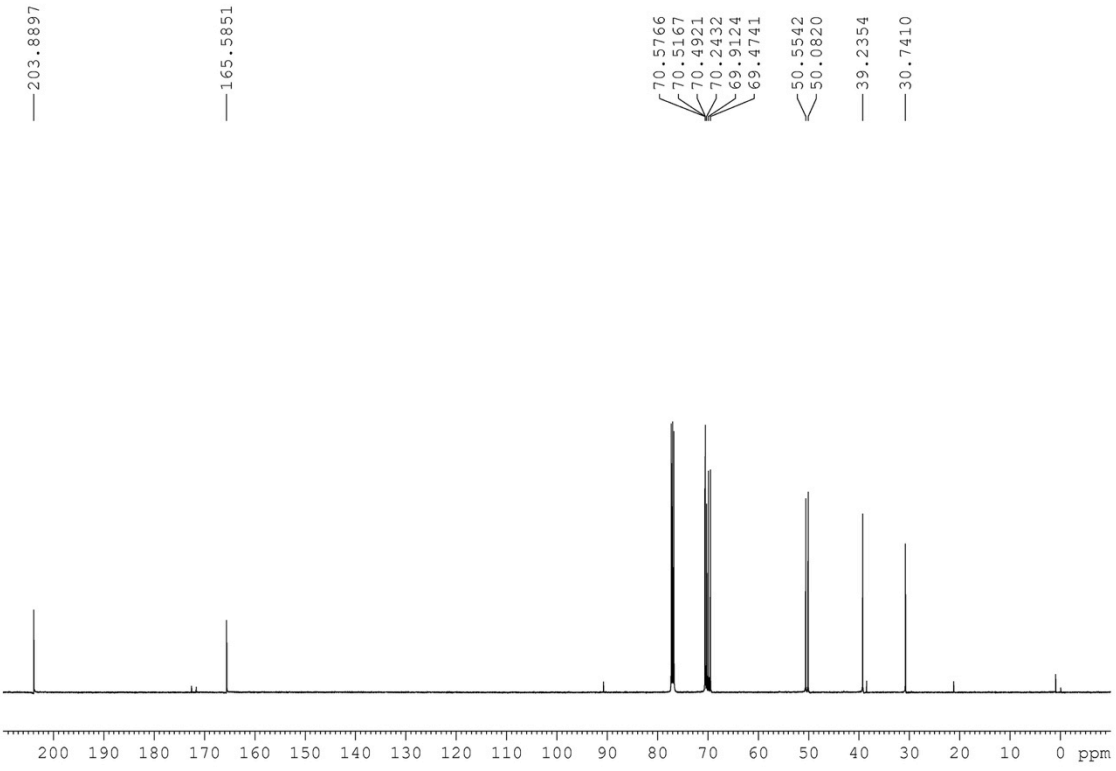




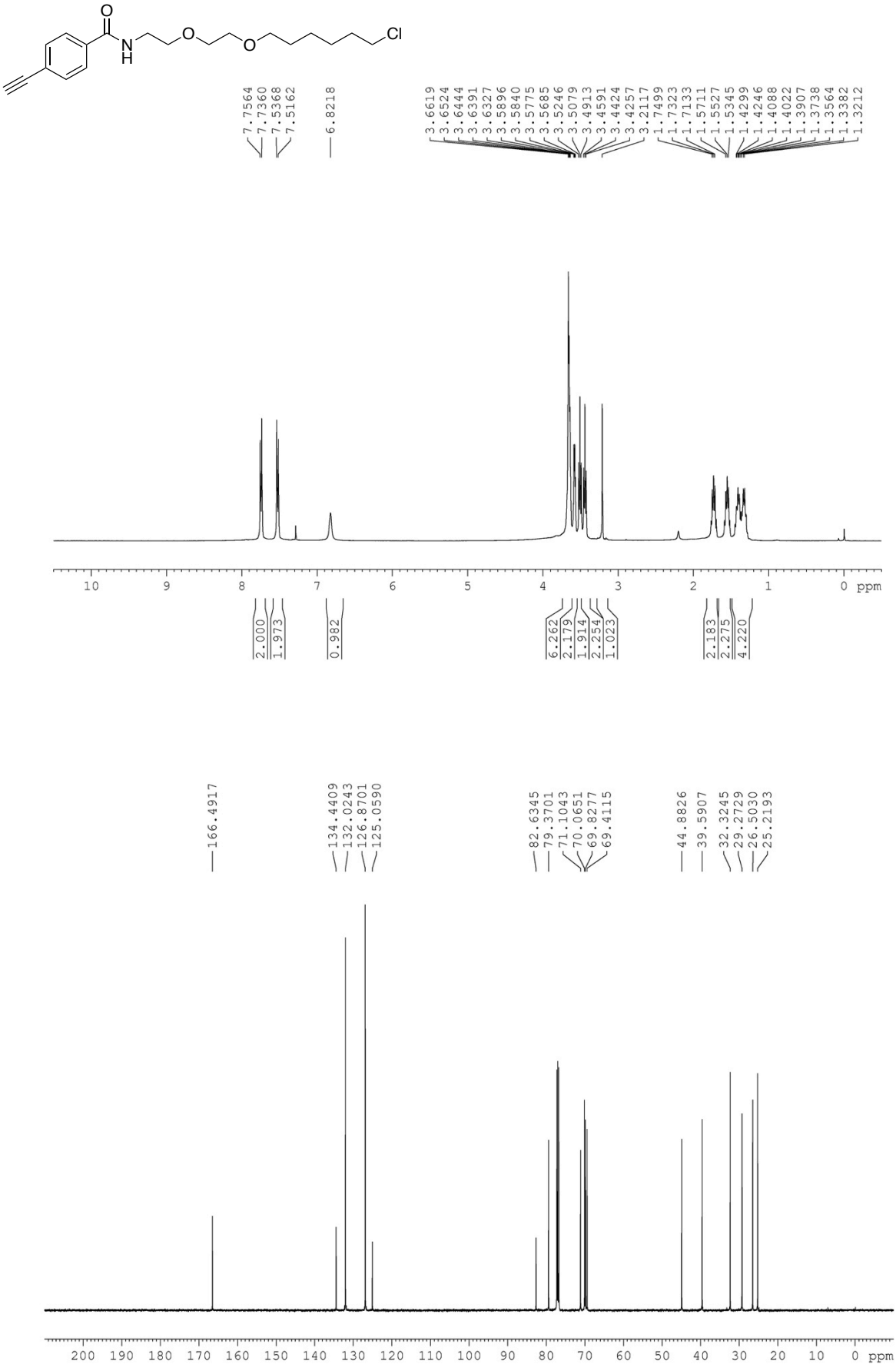
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S6** ($CDCl_3$)



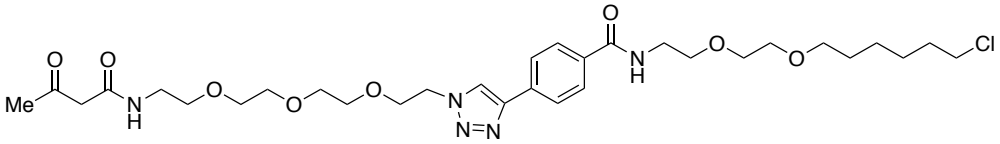


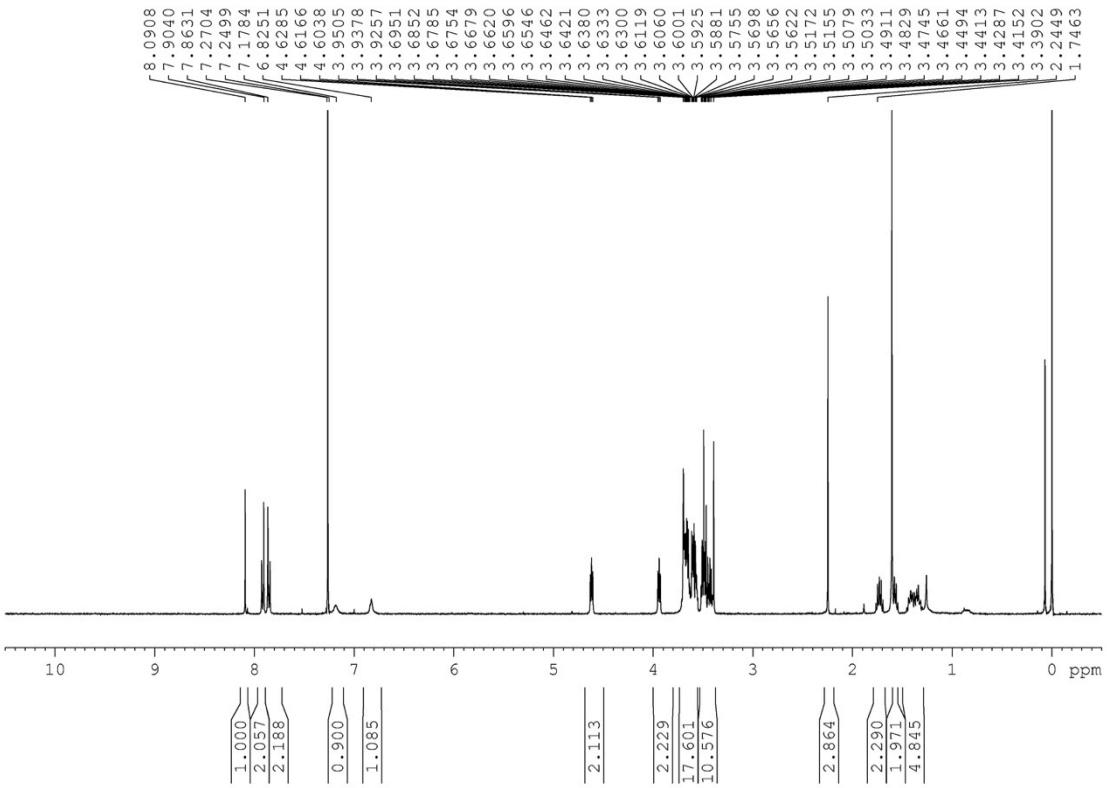


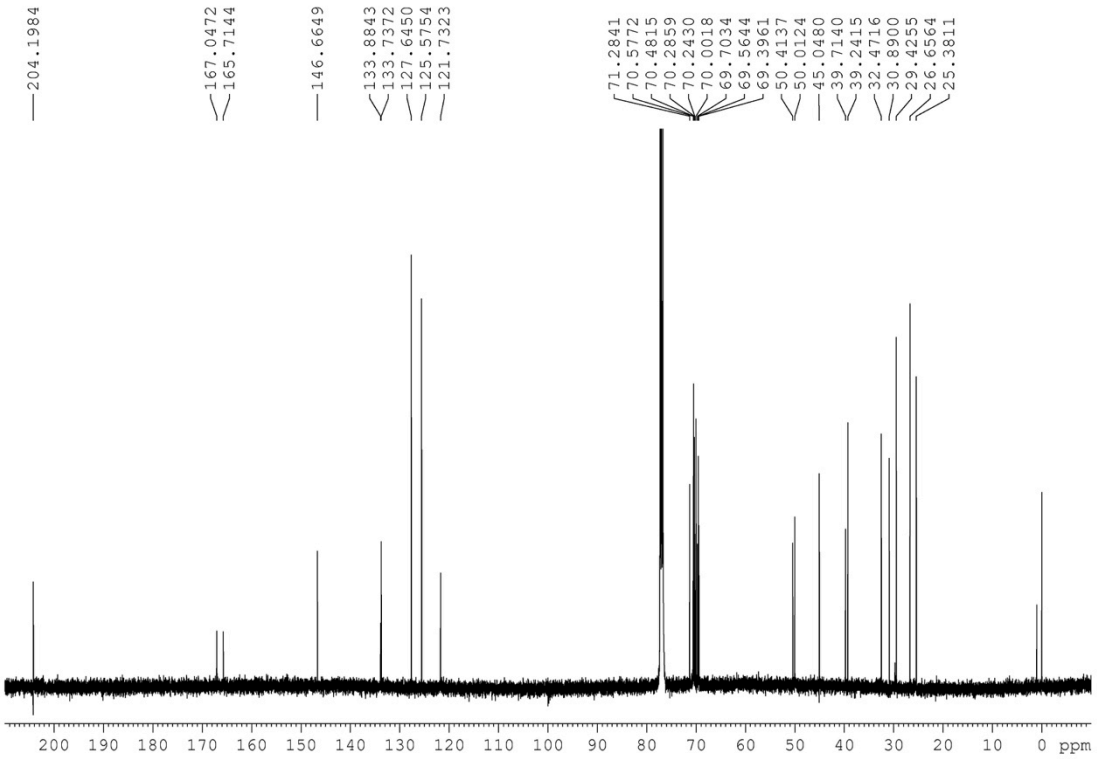
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S7** ($CDCl_3$)



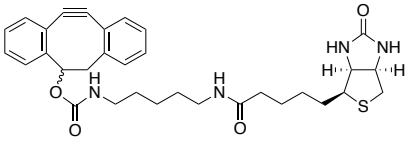
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **13b** ($CDCl_3$)

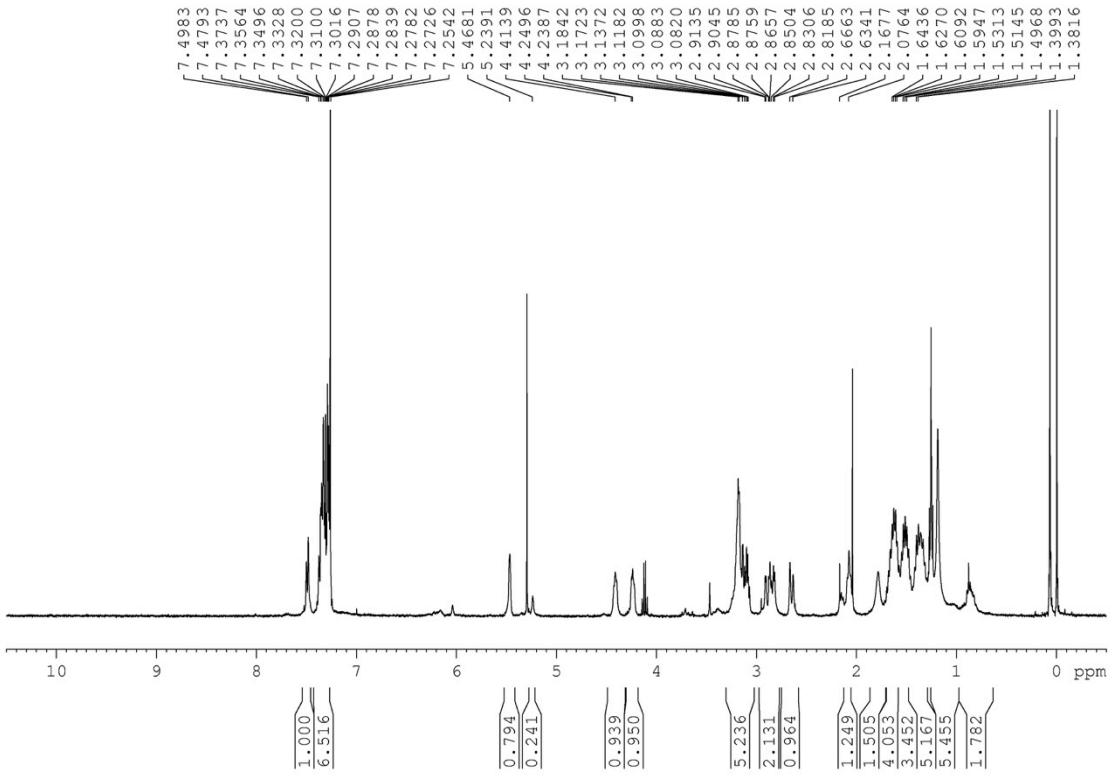


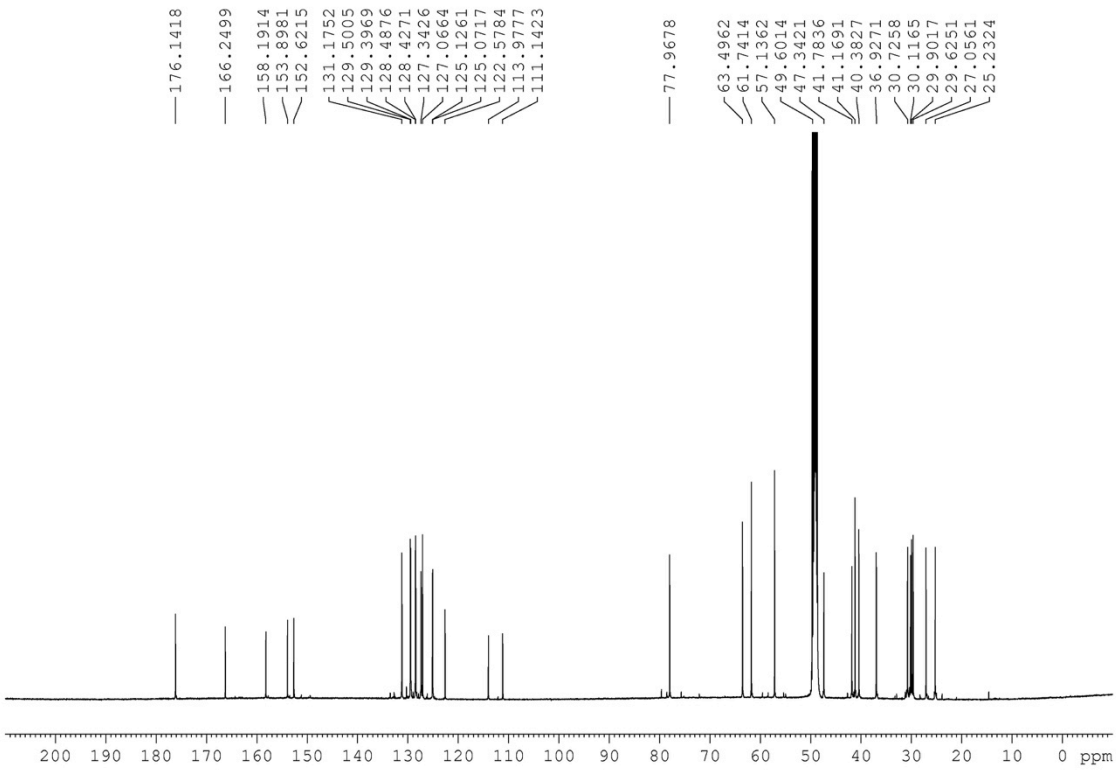




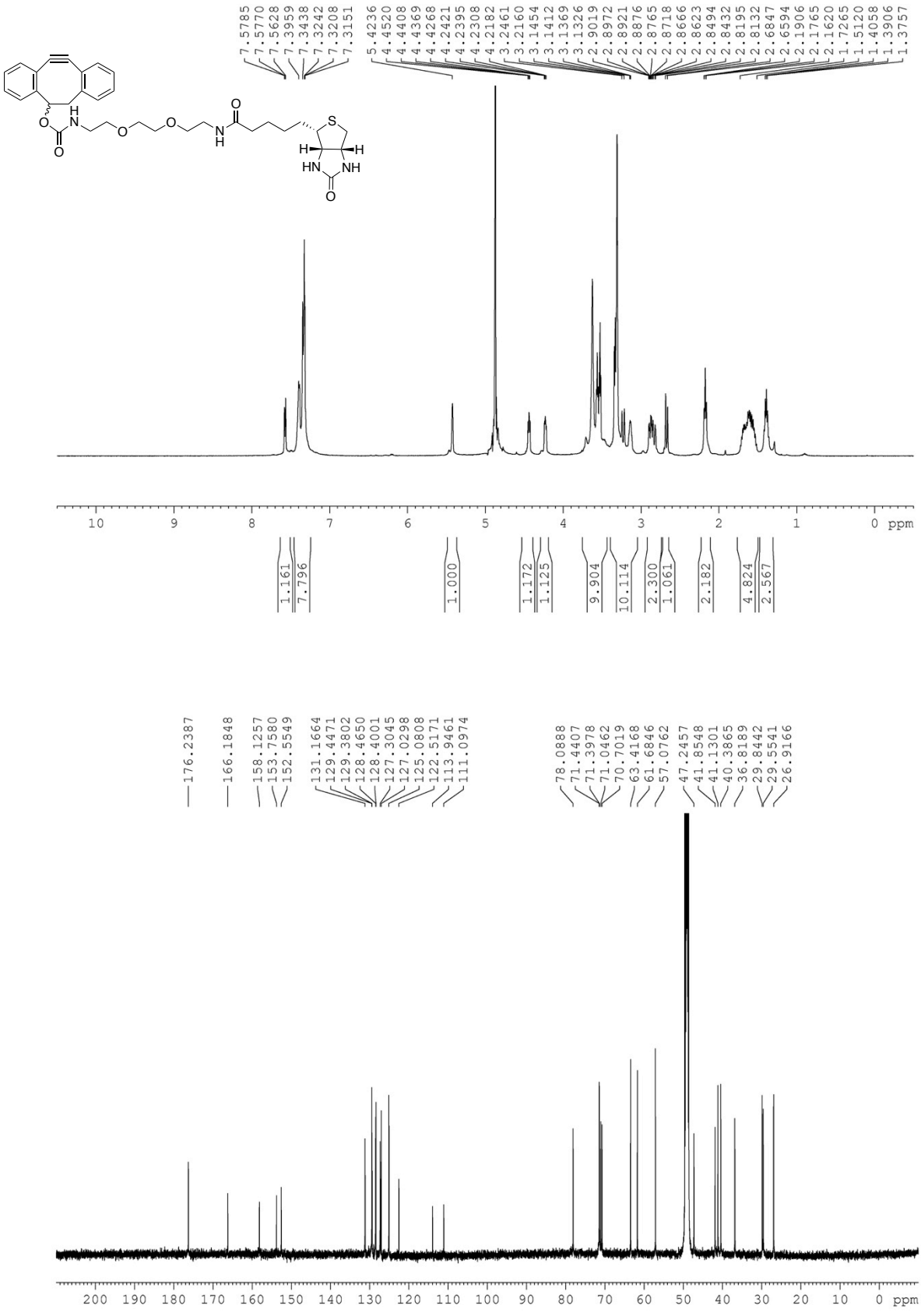
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (126 MHz, CD_3OD) spectra of **15a**



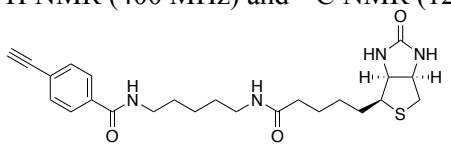


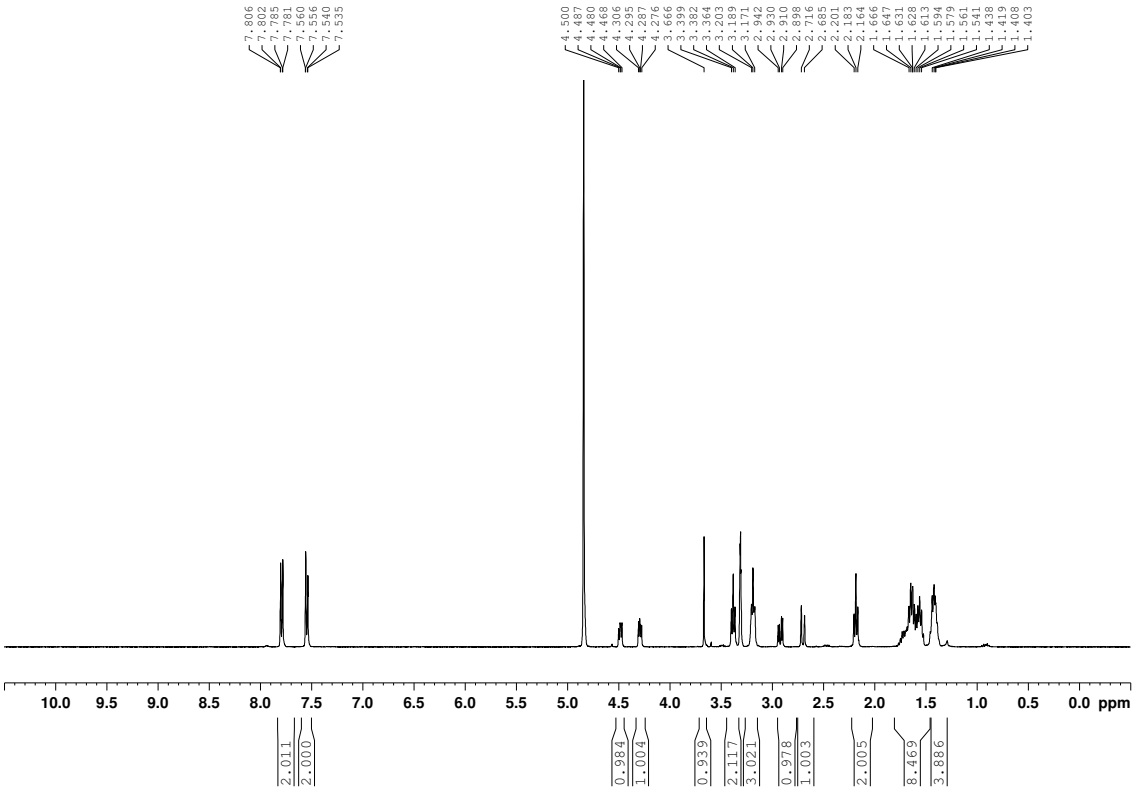


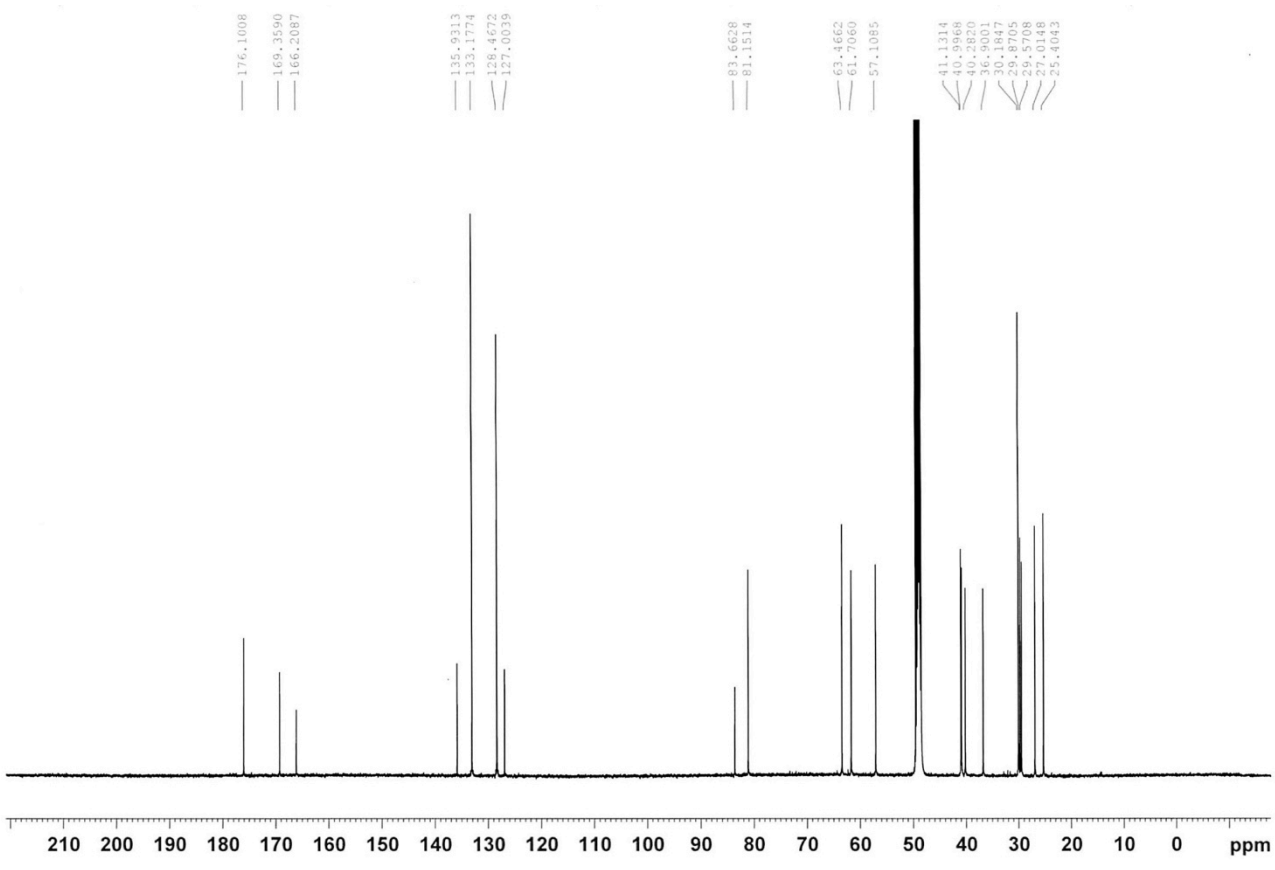
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **15b** (CD_3OD)



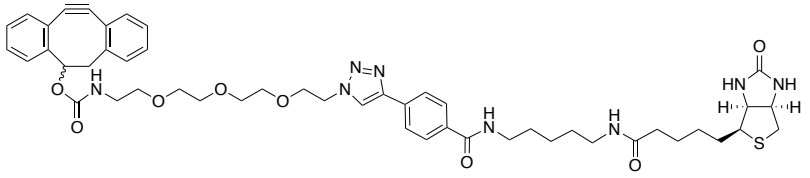
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S8** (CD_3OD)

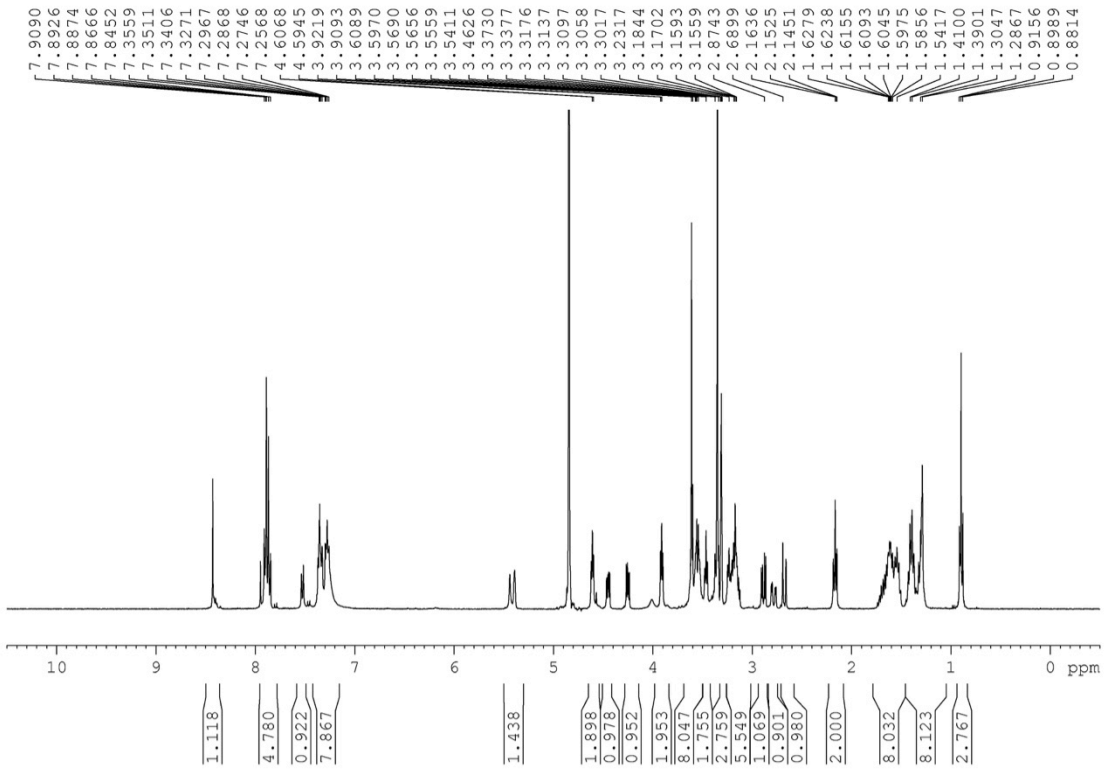


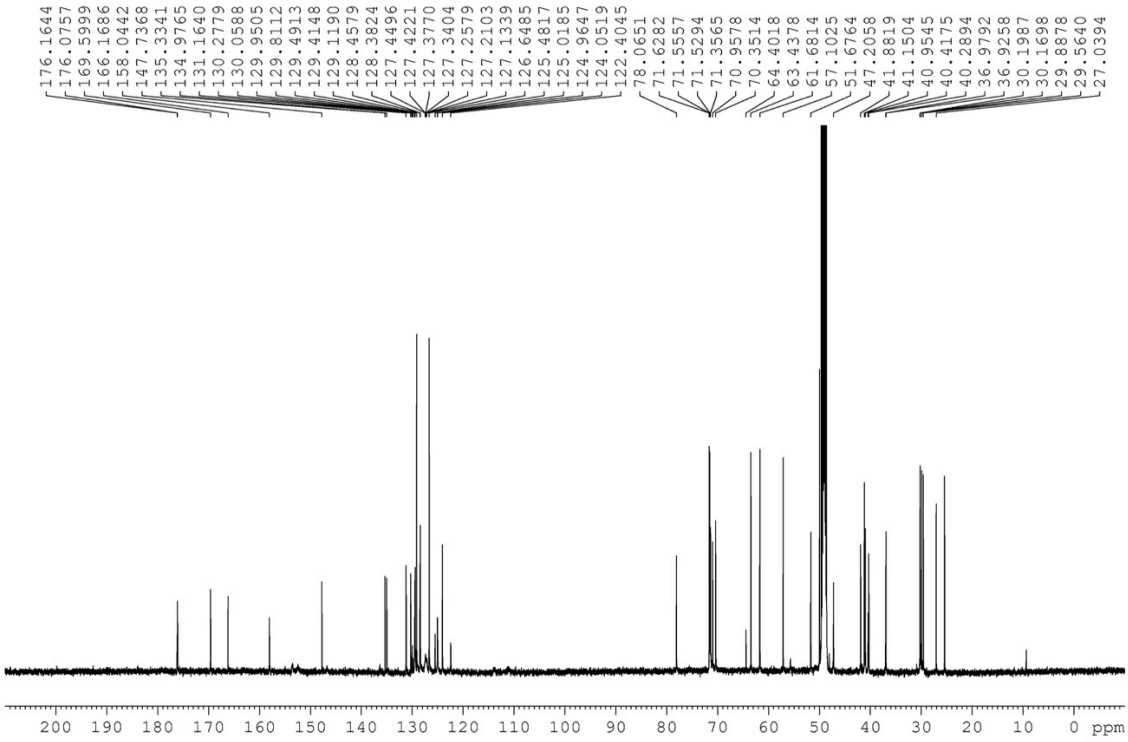




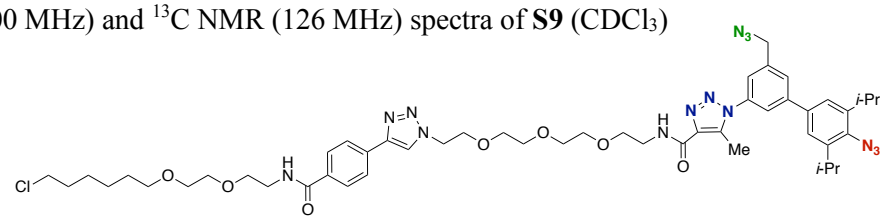
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **15c** (CD_3OD)

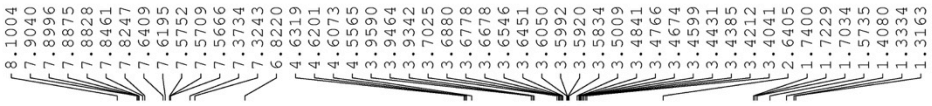


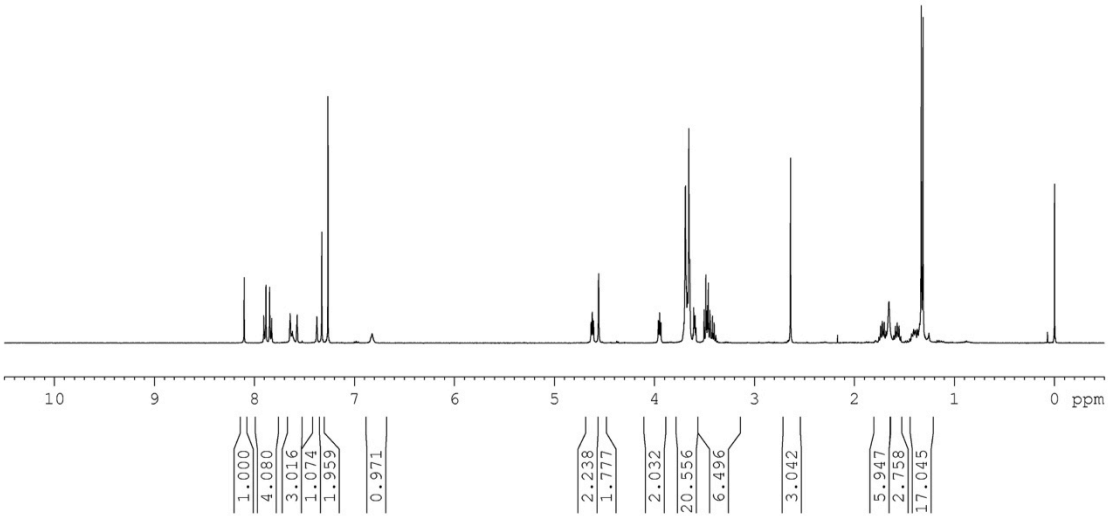


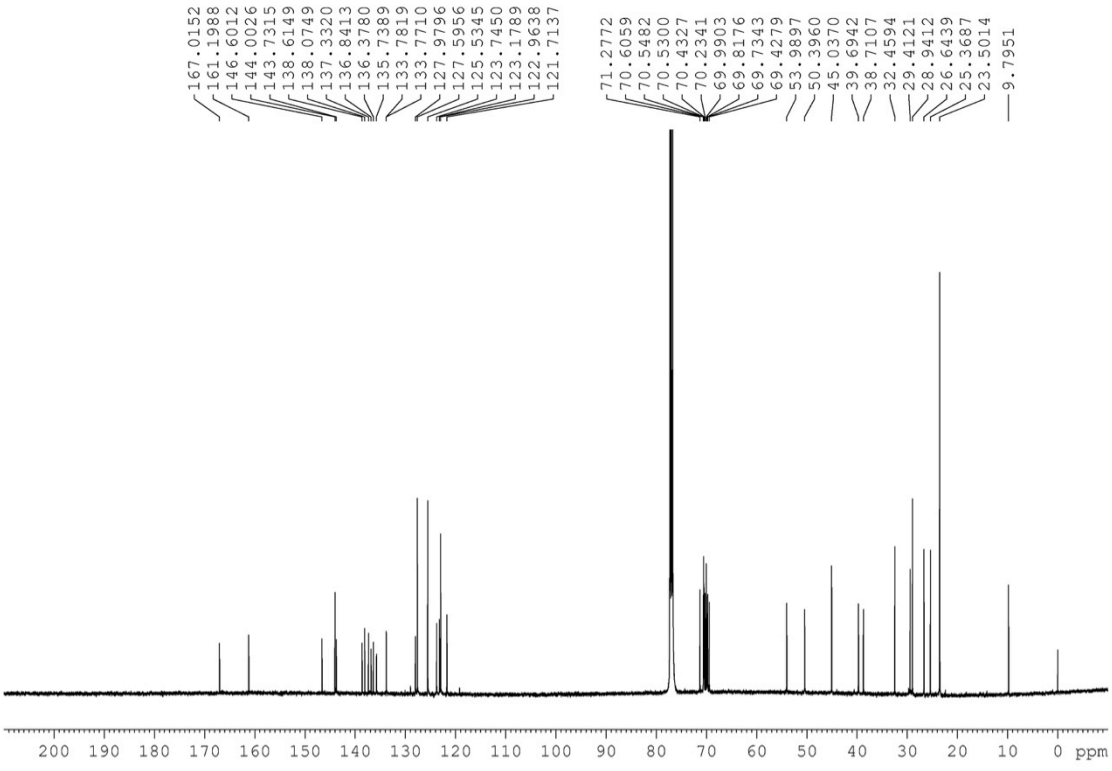


^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S9** ($CDCl_3$)

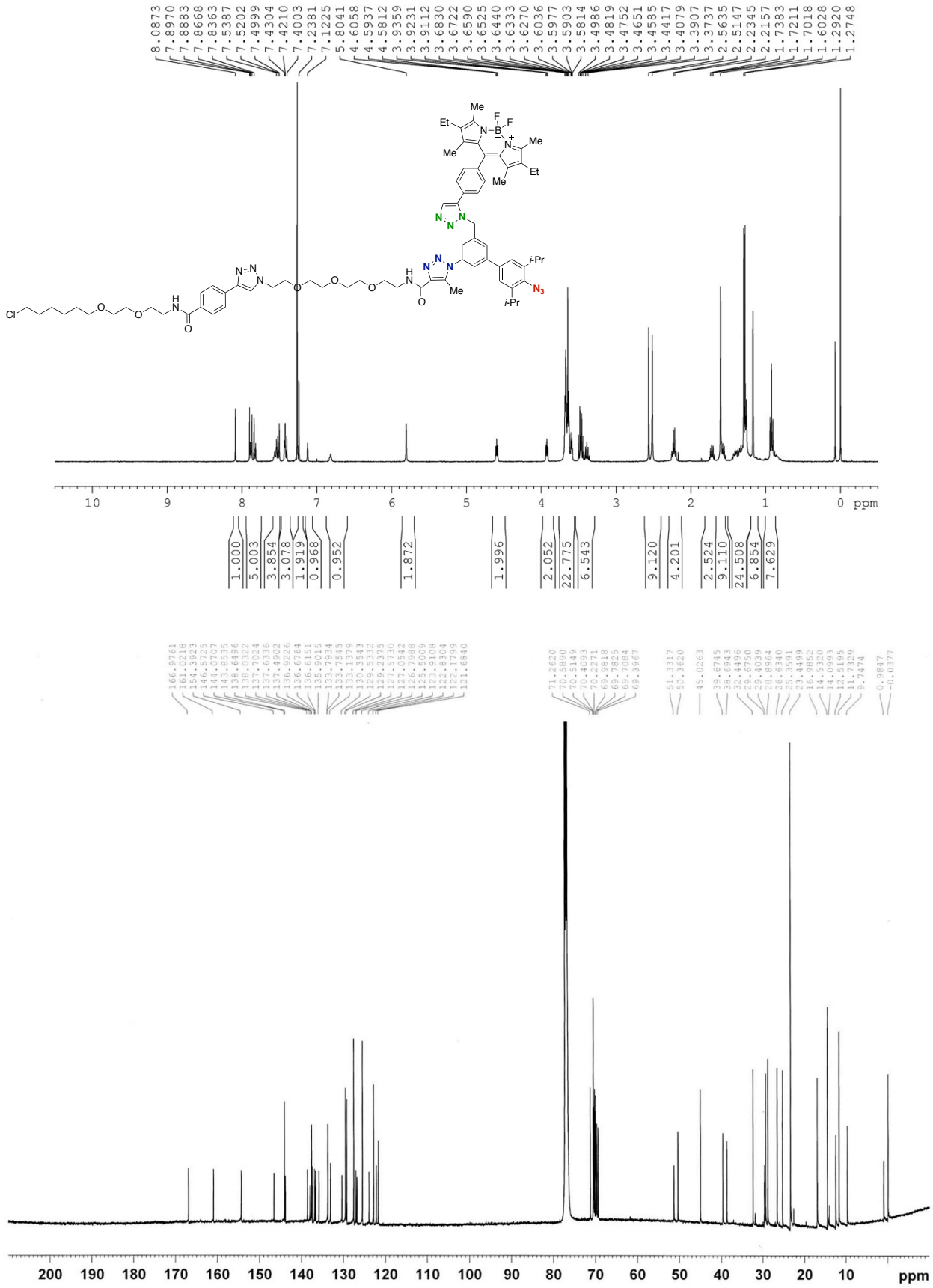




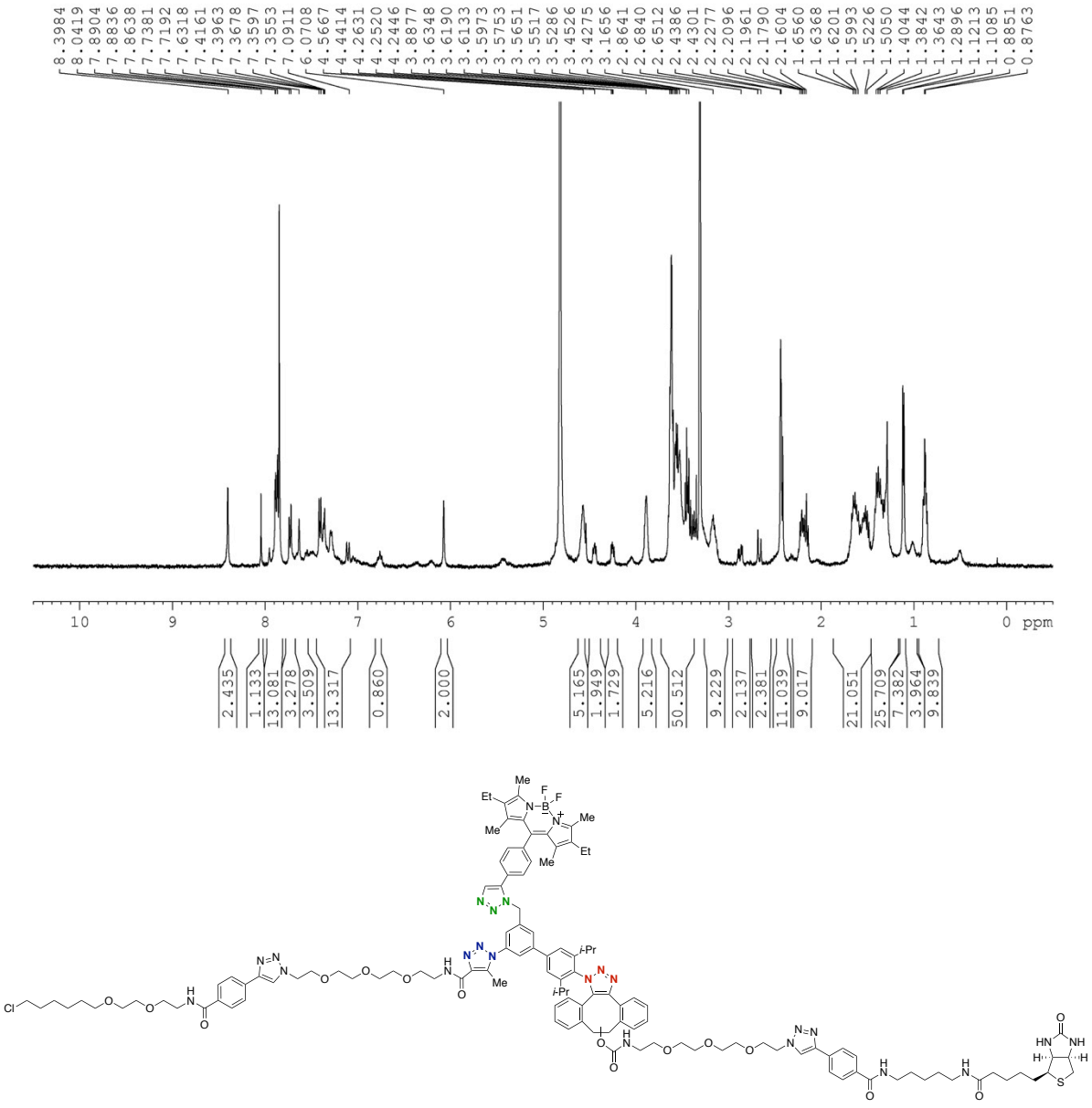




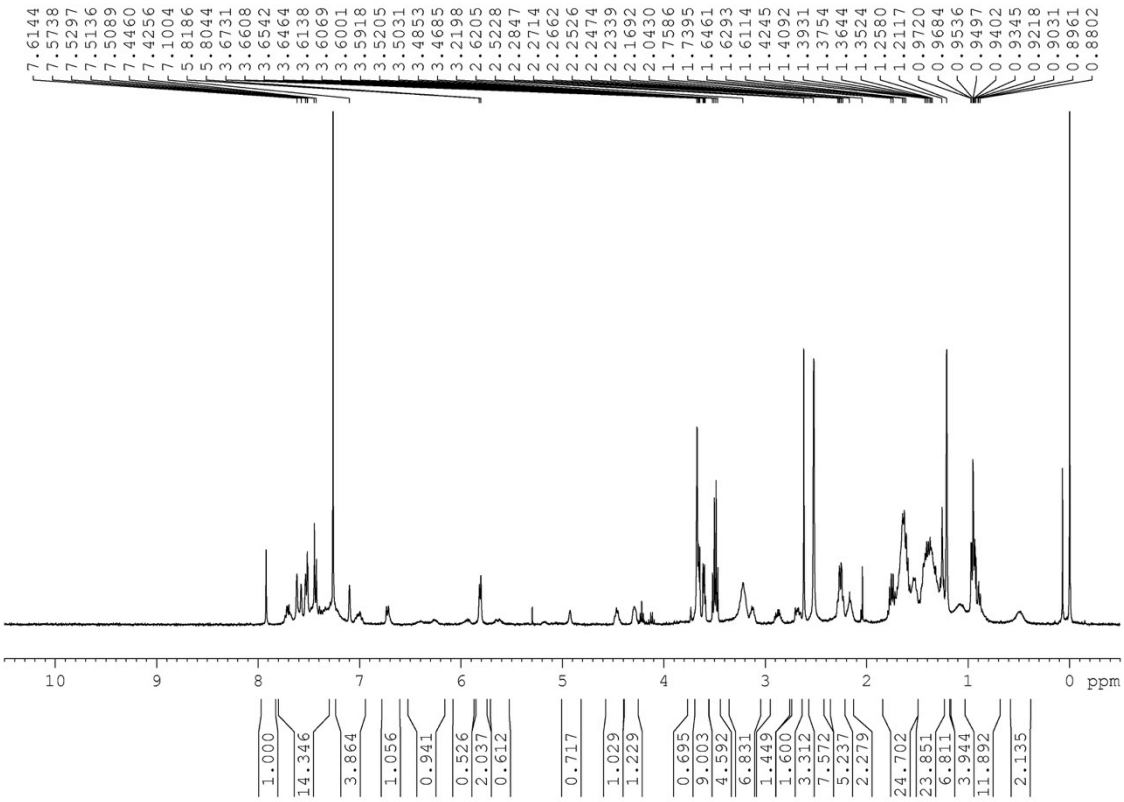
^{1}H NMR (400 MHz) and ^{13}C NMR (126 MHz) spectra of **S10** ($CDCl_3$)

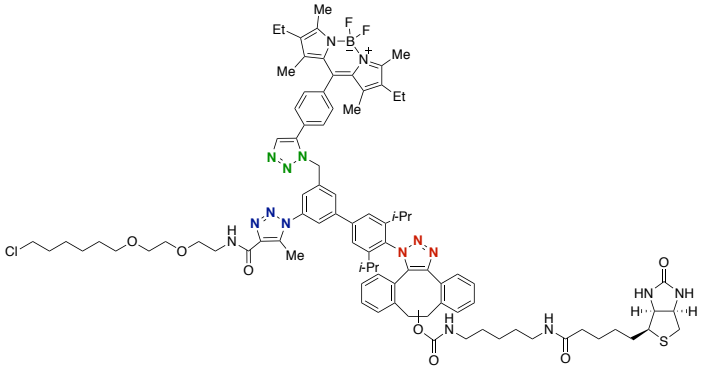


^{1}H NMR (400 MHz) spectrum of **16d** (CD_3OD)

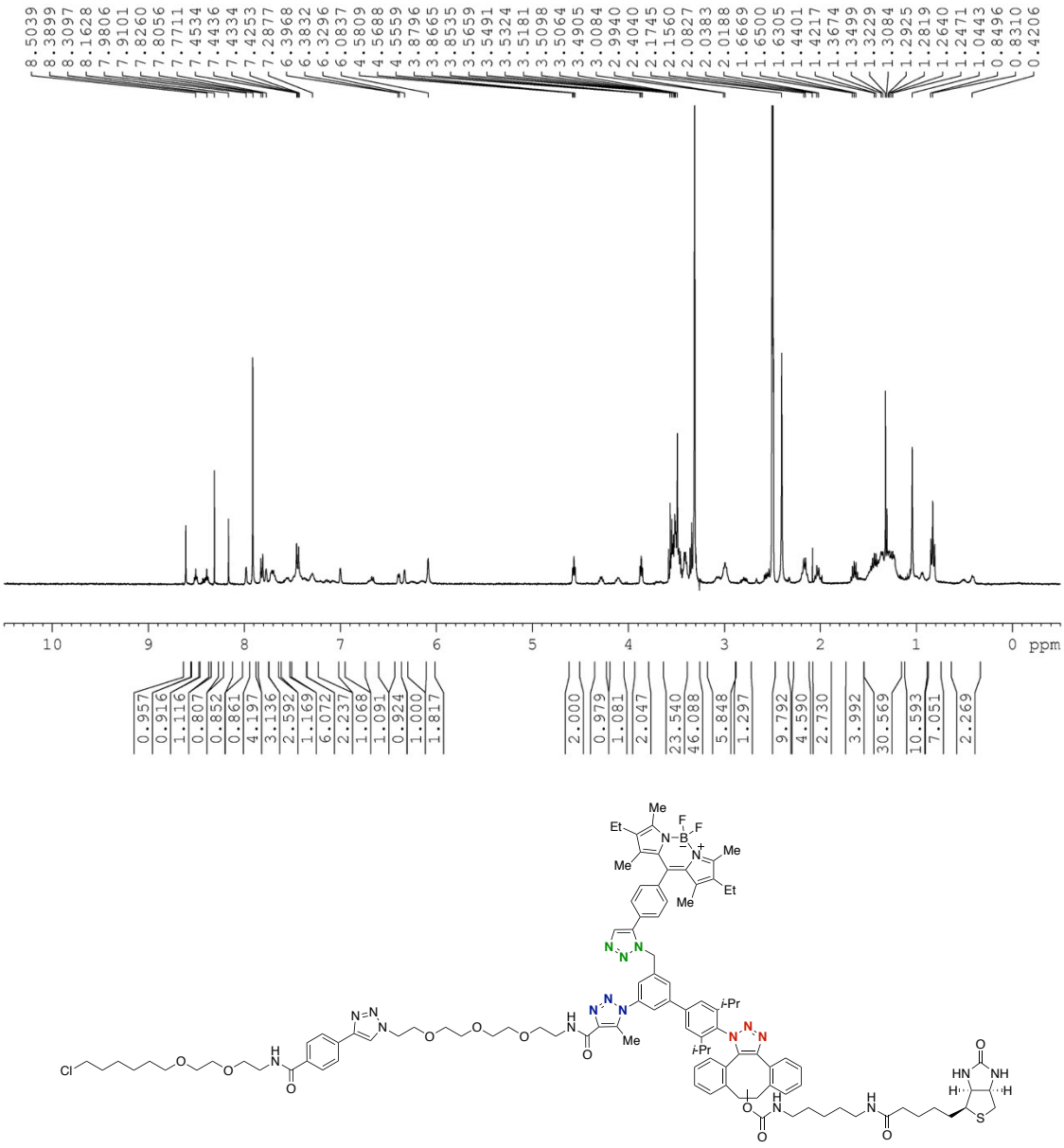


^{1}H NMR (400 MHz) spectrum of **16a** (DMSO-d_6)

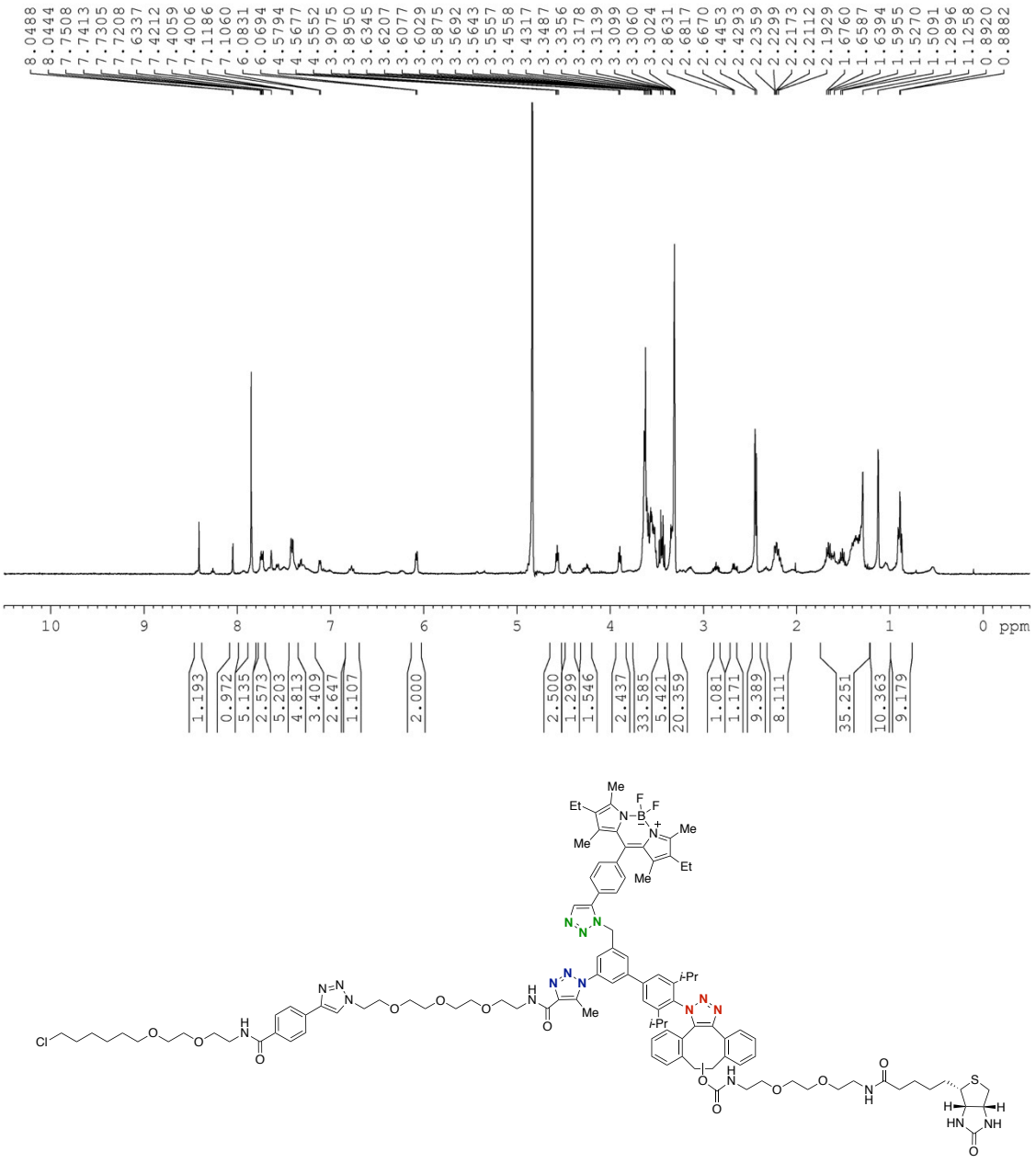




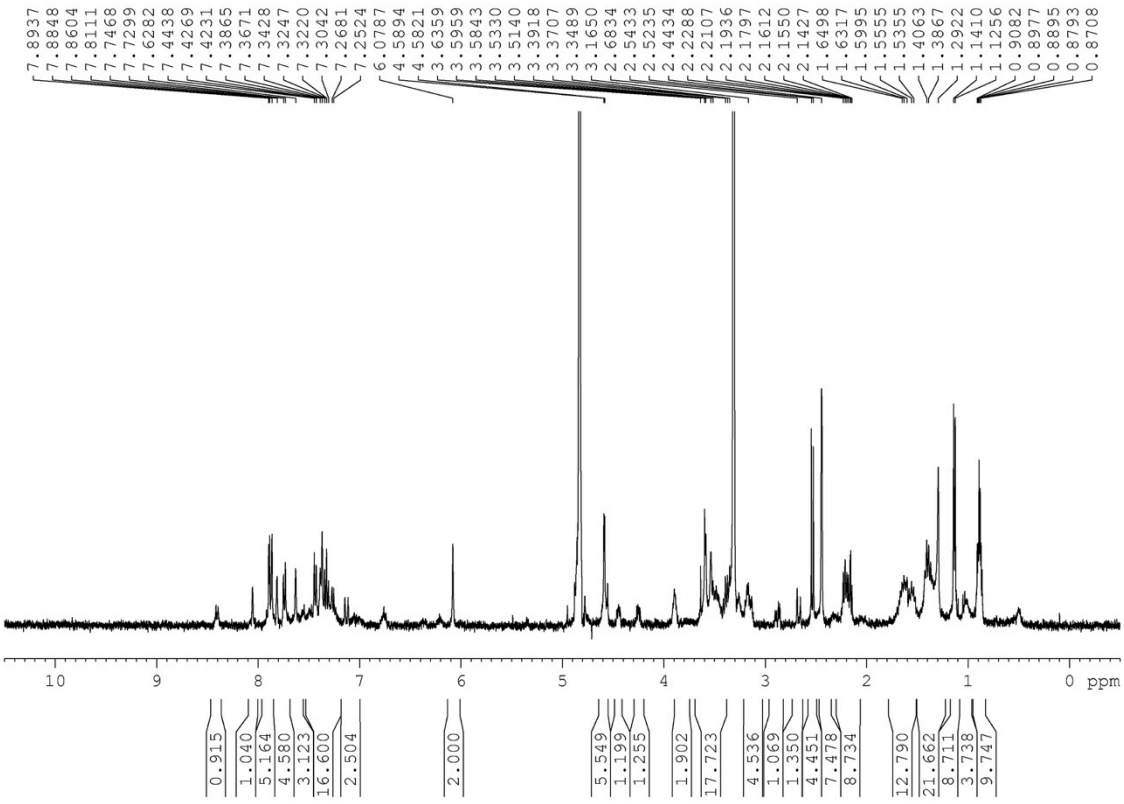
^{1}H NMR (400 MHz) spectrum of **16b** (DMSO-d_6)

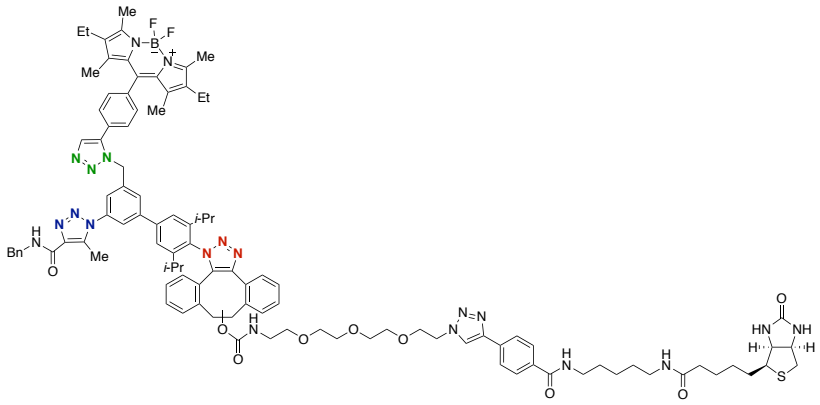


^{1}H NMR (400 MHz) spectrum of **16c** (CD_3OD)

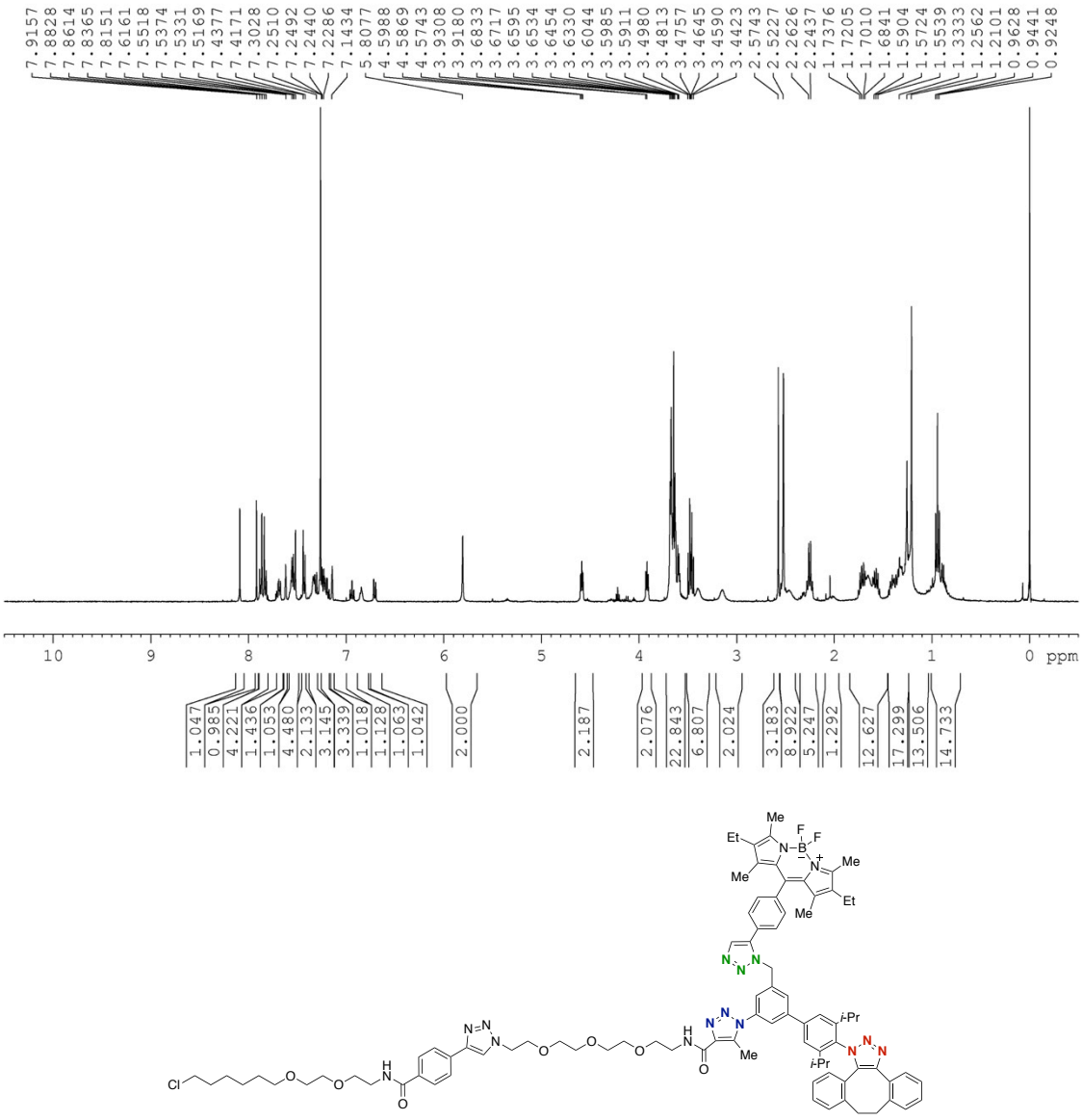


^{1}H NMR (400 MHz) spectrum of **S11** (CD_3OD)





^{1}H NMR (400 MHz) spectrum of **S12** ($CDCl_3$)



^{1}H NMR (400 MHz) spectrum of **S13** (CD_3OD)