Supporting Information

Facile assembly of three cycloalkyne-modules onto a platform compound bearing thiophene *S*,*S*-dioxide moiety and two azido groups

Tomohiro Meguro, Yuki Sakata, Takamoto Morita, Takamitsu Hosoya, and Suguru Yoshida*

Laboratory of Chemical Bioscience, Institute of Biomaterials and Bioengineering,
Tokyo Medical and Dental University (TMDU),
2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan

Contents

General Remarks

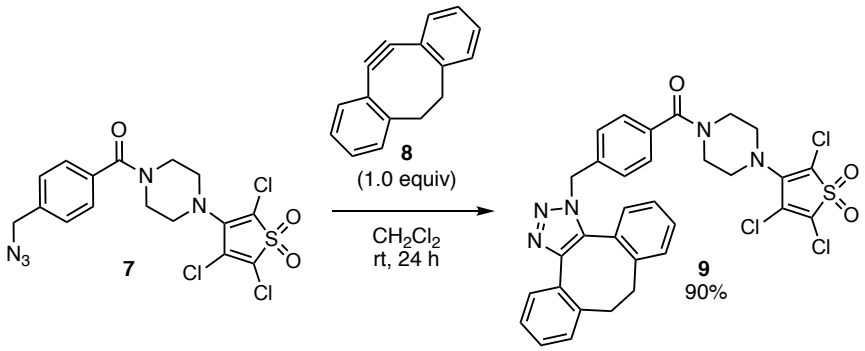
All reactions were performed with dry glassware under atmosphere of argon, unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm) silica-gel plates (Merck Chemicals, Silica Gel 60 F254, Cat. No. 105715). Column chromatography was conducted using silica-gel (Kanto Chemical Co., Inc., Silica Gel 60N, spherical neutral, particle size 40–50 μm). Preparative thin-layer chromatography (PTLC) was performed on silica-gel (Wako Pure Chemical Industries Ltd., Wakogel B5-F, Cat. No. 230-00043). Melting points (Mp) were measured on an OptiMelt MPA100 (Stanford Research Systems), and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker AVANCE 500 spectrometer at 500 MHz. ^{13}C NMR spectra were obtained with a Bruker AVANCE 500 spectrometer at 126 MHz. $CDCl_3$ (Kanto Chemical Co., Inc, Cat. No.07663-23) 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 77.0 for ^{13}C NMR in $CDCl_3$) as an internal reference with coupling constants (*J*) in hertz (Hz). The abbreviations s, d, t, q, m, and br signify singlet, doublet, triplet, quartet, multiplet, and broad, respectively. IR spectra were measured by diffuse reflectance method on a Shimadzu IRPrestige-21 spectrometer attached with DRS-8000A with the absorption band given in cm^{-1}. High-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. High-resolution mass spectra (HRMS) were measured on a Bruker micrOTOF mass spectrometer under positive electrospray ionization (ESI^+).

Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. 3-(4-(4-(Azidomethyl)benzoyl)piperazin-1-yl)-2,4,5-trichlorothiophene *S*,*S*-dioxide (**7**),[S1] 5,6-Didehydro-11,12-dihydrodibenzo[*a*,*e*]cyclooctene (**8**),[S2] cyclohexyl azide (**10b**),[S3] 2-azidoadamantane (**10c**),[S4] 3-(4-(tert-butoxycarbonyl)piperazin-1-yl)-2,4,5-trichlorothiophene *S*,*S*-dioxide (**12**),[S1] 4,8-ditosyl-4,8-diazacyclononyne (**14**),[S5] 3-(azidomethyl)-5-(methoxycarbonyl)aniline (**18**),[S6] 3-azidoadamantane-1-carboxylic acid (**19**),[S7] 2,3,4,5-tetrachlorothiophene *S*,*S*-dioxide (**23**),[S8] (1*α*,8*α*,9*α*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**25**),[S9] 1-(2-(2-(2-(2-(4-(3,6-bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido)ethoxy)ethoxy)ethoxy)ethyl)-4-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropylaminocarbonyloxymethyl)-1*H*-[1,2,3]triazole (**28a**),[S10] 1-(2-(2-(2-(2-(biotinamido)ethoxy)ethoxy)ethoxy)ethyl)-4-(3-(4-tosyl-4,8-diazacyclononyn-8-ylcarbonyl)propionylaminomethyl)-1*H*-[1,2,3]triazole (**28b**),[S10] 4-((1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethoxycarbonylaminomethyl)-1-(4-(2-(2-(6-chlorohexoxy)ethoxy)ethylaminocarbonyl)benzyl)-1*H*-[1,2,3]triazole (**28c**)[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.***

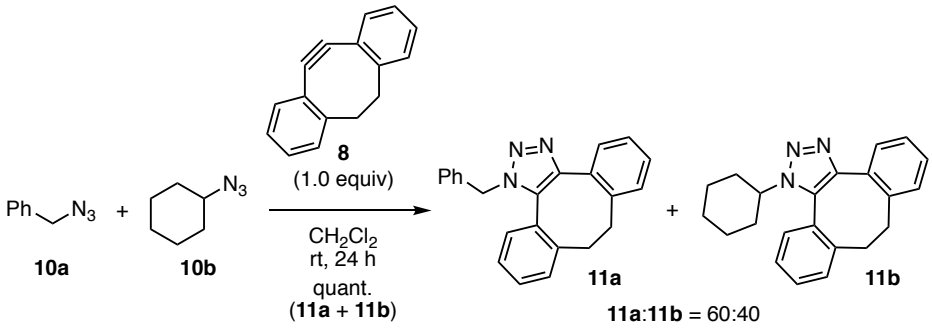
Experimental Procedures

Conjugation of thiophene dioxide 7 with cyclooctyne ***8***



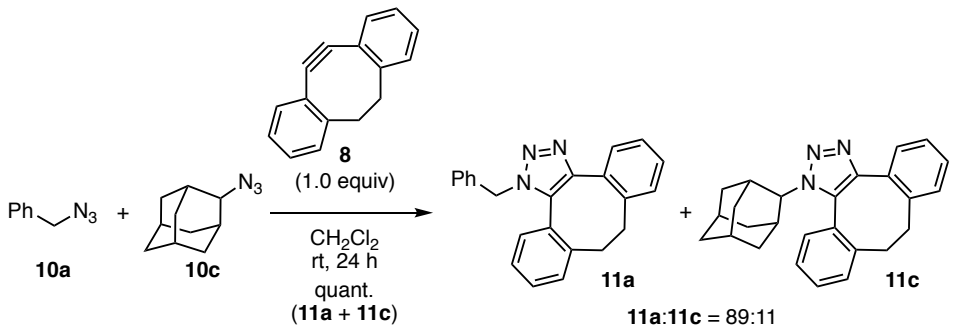
To a solution of 3-(4-(4-(azidomethyl)benzoyl)piperazin-1-yl)-2,4,5-trichlorothiophene *S*,*S*-dioxide (**7**) (23.1 mg, 49.9 μmol) dissolved in CH_2Cl_2 (1.0 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a*,*e*]cyclooctene (**8**) (10.1 mg, 49.4 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (EtOAc/*n*-hexane = 3/1) to give 3-(4-(4-(8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazol-1-yl)methyl)benzoyl)piperazin-1-yl)-2,4,5-tetrachlorothiophene *S*,*S*-dioxide (**9**) (29.8 mg, 44.7 μmol, 90%) as a yellow solid.

Competition experiment using azides ***10a*** *and* ***10b*** *with cyclooctyne* ***8***



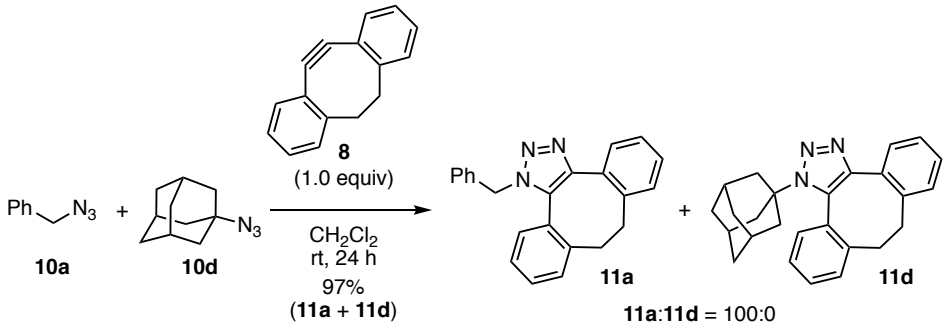
To a mixture of benzyl azide (**10a**) (13.2 mg, 99.1 μmol) and cyclohexyl azide (**10b**) (12.4 mg, 99.1 μmol) dissolved in CH_2Cl_2 (1.0 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a*,*e*]cyclooctene (**8**) (20.2 mg, 98.9 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. To the residue was added 1,1,2,2-tetrachloroethane (18.0 mg, 0.104 mmol) as an internal standard, dissolved in $CDCl_3$, and ^1H NMR analysis (400 MHz) was performed. Yield of **11a** + **11b** was determined to be quantitative (**11a**:**11b** = 60:40), by comparing the relative values of integration for the peaks observed at 5.56 ppm (s, 2H) for **11a** and 4.17–4.23 (m, 1H) for **11b** with that of 1,1,2,2-tetrachloroethane observed at 5.95 ppm (s, 2H).

Competition experiment using azides ***10a*** *and* ***10c*** *with cyclooctyne* ***8***



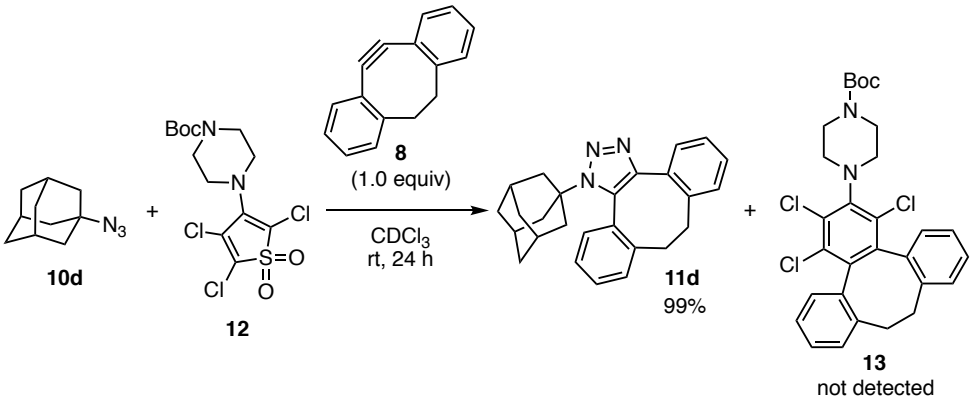
To a mixture of benzyl azide (**10a**) (13.2 mg, 99.1 μmol) and 2-azidoadamantane (**10c**) (17.7 mg, 99.9 μmol) dissolved in CH_2Cl_2 (1.0 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a*,*e*]cyclooctene (**8**) (20.2 mg, 98.9 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. To the residue was added 1,1,2,2-tetrachloroethane (16.0 mg, 92.5 μmol) as an internal standard, dissolved in $CDCl_3$, and ^{1}H NMR analysis (400 MHz) was performed. Yield of **11a** + **11c** was determined to be quantitative (**11a**:**11c** = 89:11), by comparing the relative values of integration for the peaks observed at 5.56 ppm (s, 2H) for **11a** and 4.66 ppm (s, 1H) for **11c** with that of 1,1,2,2-tetrachloroethane observed at 5.95 ppm (s, 2H).

*Competition experiment using azides **10a** and **10d** with cyclooctyne **8***



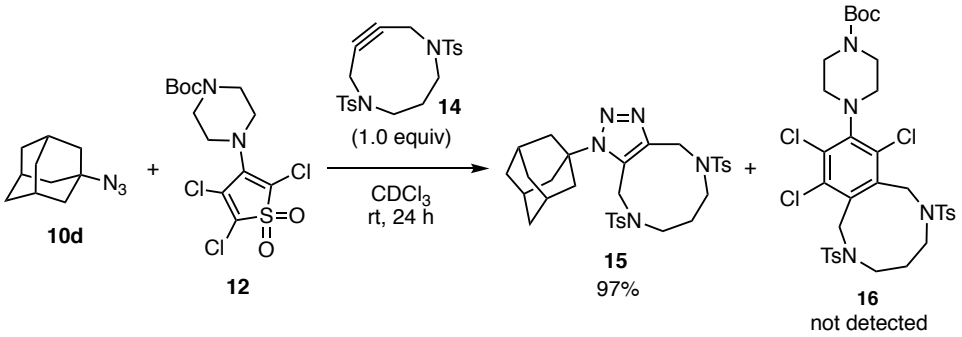
To a mixture of benzyl azide (**10a**) (13.3 mg, 99.9 μmol) and 1-azidoadamantane (**10d**) (17.8 mg, 0.100 mmol) dissolved in CH_2Cl_2 (1.0 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a*,*e*]cyclooctene (**8**) (20.2 mg, 98.9 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. To the residue was added 1,1,2,2-tetrachloroethane (15.7 mg, 90.7 μmol) as an internal standard, dissolved in $CDCl_3$, and ^{1}H NMR analysis (400 MHz) was performed. Yield of **11a** + **11d** was determined to be 97% (**11a**:**11d** = 100:0), by comparing the relative values of integration for the peaks observed at 5.56 ppm (s, 2H) for **11a** with that of 1,1,2,2-tetrachloroethane observed at 5.95 ppm (s, 2H).

*Competition experiment using azide **10d** and thiophene dioxide **12** with cyclooctyne **8***



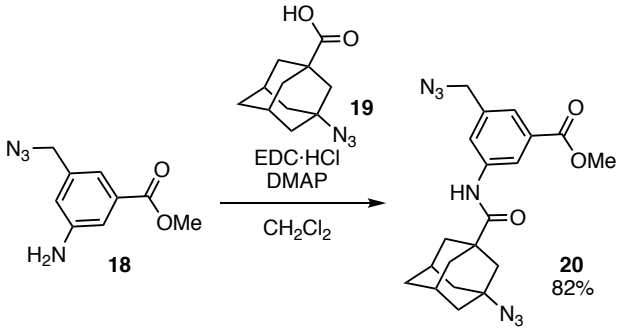
To a mixture of 1-azidoadamantane (**10d**) (13.4 mg, 75.6 μmol), 3-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2,4,5-trichlorothiophene *S*,*S*-dioxide (**12**) (30.3 mg, 75.1 μmol) and 1,1,2,2-tetrachloroethane (18.3 mg, 0.106 mmol) as an internal standard, dissolved in $CDCl_3$ (1.0 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a*,*e*]cyclooctene (**8**) (15.2 mg, 74.4 μmol) dissolved in $CDCl_3$ (0.50 mL) at room temperature. After stirring for 24 h at the same temperature, the mixture was transferred into an NMR tube, and the ^{1}H NMR analysis (500 MHz) was performed. Yields of **11d** and **13** was determined to be 99% and 0% by comparing the relative values of integration for the peaks observed at 7.40–7.42 (m, 1H) for **11d** with that of 1,1,2,2-tetrachloroethane observed at 5.95 ppm (s, 2H).

*Competition experiment using azide **10d** and thiophene dioxide **12** with cycloalkyne **14***



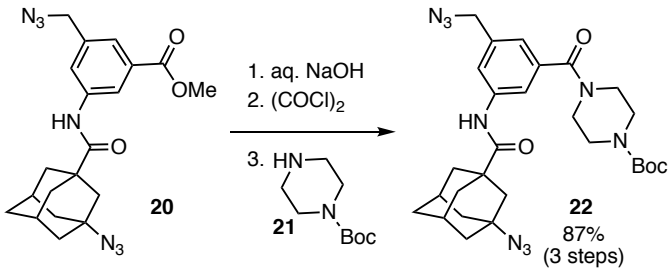
To a mixture of 1-azidoadamantane (**10d**) (13.4 mg, 75.1 μmol), 3-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2,4,5-trichlorothiophene *S*,*S*-dioxide (**12**) (30.3 mg, 75.6 μmol) and 1,1,2,2-tetrachloroethane (17.4 mg, 0.101 mmol) as an internal standard, dissolved in $CDCl_3$ (1.0 mL) was added 4,8-ditosyl-4,8-diazacyclononyne (**14**) (32.4 mg, 74.9 μmol) dissolved in $CDCl_3$ (0.50 mL) at room temperature. After stirring for 24 h at the same temperature, the mixture was transferred into an NMR tube, and the ^{1}H NMR analysis (400 MHz) was performed. Yields of **15** and **16** was determined to be 97% and 0% by comparing the relative values of integration for the peaks observed at 4.92 (s, 2H) for **15** with that of 1,1,2,2-tetrachloroethane observed at 5.95 ppm (s, 2H).

*Synthesis of 1-(3-azidoadamantane-1-carboxamido)-3-azidomethyl-5-methoxycarbonylbenzene (**20**)*



To a mixture of compound **18** (136 mg, 0.660 mmol) and 3-azidoadamantane-1-carboxylic acid (**19**) (171 mg, 0.773 mmol) dissolved in CH_2Cl_2 (5.0 mL) was added 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC·HCl) (153 mg, 0.798 mmol) and 4-(dimethylamino)pyridine (DMAP) (94.0 mg, 0.769 mmol) at room temperature. After stirring for 40 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was added Et_2O (20 mL) and H_2O (20 mL). The mixture was extracted with Et_2O (20 mL × 2), and the combined organic extract was washed with brine (20 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica-gel 10 g, *n*-hexane/EtOAc = 1/1) to give 1-(3-azidoadamantane-1-carboxamido)-3-azidomethyl-5-methoxycarbonylbenzene (**20**) (221 mg, 0.540 mmol, 82%) as a colorless oil.

*Synthesis of 1-(3-azidoadamantane-1-carboxamido)-3-azidomethyl-5-(4-(tert-butoxycarbonyl) piperazin-1-yl)carbonylbenzene **22***



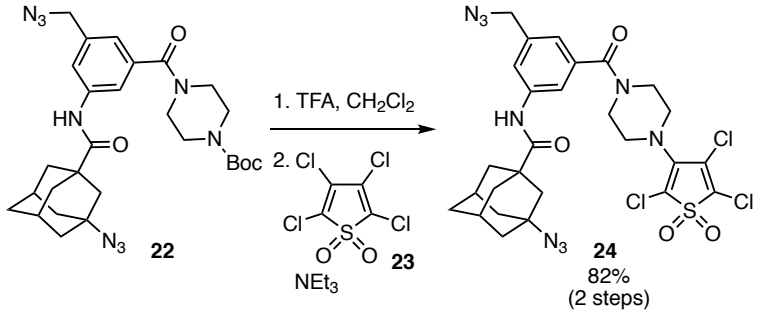
To a solution of compound **20** (227 mg, 0.554 mmol) in THF (2.0 mL) and MeOH (2.0 mL) was added aqueous 1.0 M NaOH (1.0 mL) at 0 °C. After gradually warming to room temperature, the mixture was stirred for 14 h, and to this was added 1 M HCl. The mixture was extracted with EtOAc (20 mL × 3), and the combined

organic extract was washed with brine (20 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure.

Without further purification the carboxylic acid was dissolved in CH_2Cl_2 (2.0 mL) and DMF (1 drop). To the solution was added $(COCl)_2$ (86.0 μL, 1.00 mmol) at 0 °C. After gradually warming to room temperature, the mixture was stirred for 2 h, and then concentrated under reduced pressure.

Without further purification the benzoyl chloride was dissolved in CH_2Cl_2 (2.0 mL). To the solution was added *N*-Boc-piperazine **21** (398 mg 2.14 mmol) at room temperature, and the mixture was stirred for 12 h at the same temperature. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography (silica-gel 20 g, EtOAc/*n*-hexane = 2/1 to 3/1) to give 1-(3-azidoadamantane-1-carboxamido)-3-azidomethyl-5-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)carbonylbenzene (**22**) (270 mg, 0.479 mmol, 87% in 3 steps from **20**) as a colorless amorphous solid.

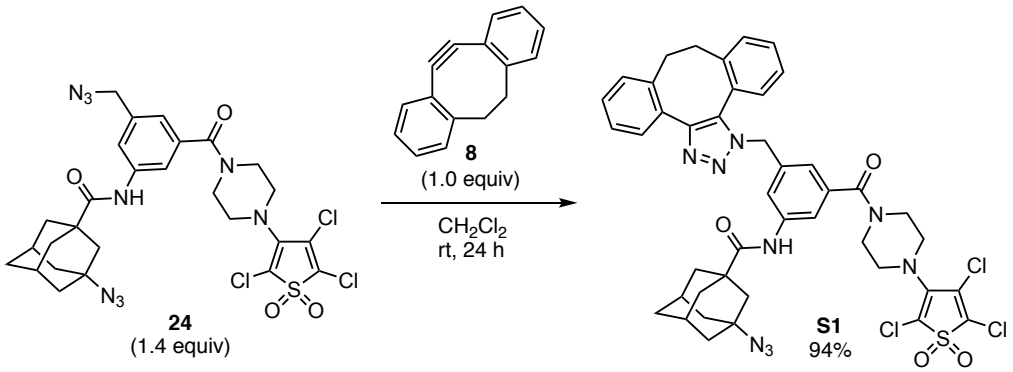
*Synthesis of platform **24***



To a solution of compound **22** (46.3 mg, 82.1 μmol) dissolved in CH_2Cl_2 (1.0 mL) was carefully added trifluoroacetic acid (0.70 mL) at 0 °C. After gradually warming to room temperature, the mixture was stirred for 7 h. After cooling down to 0 °C, aqueous saturated $NaHCO_3$ (20 mL) was added the reaction mixture. The mixture was extracted with CH_2Cl_2 (20 mL × 3), and the combined organic extract was washed with brine (20 mL), dried (Na_2SO_4), and after filtration, the filtrate was concentrated under reduced pressure.

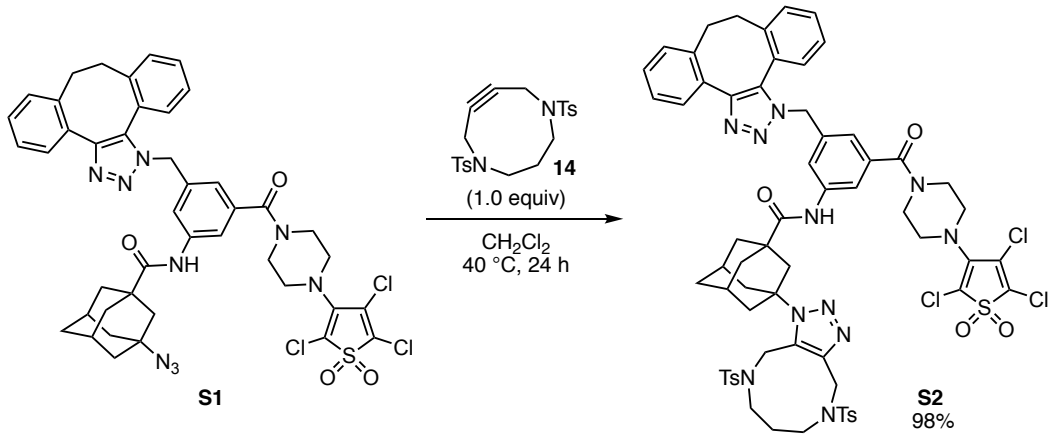
Without further purification the resulting mixture was dissolved in CH_2Cl_2 (1.0 mL). To the solution was added 2,3,4,5-tetrachlorothiophene *S*,*S*-dioxide (**23**) (60.9 mg, 0.240 mmol) dissolved in CH_2Cl_2 (1.0 mL) and triethylamine (11.0 μL, 78.7 μmol) at room temperature. After stirring for 6 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (EtOAc/*n*-hexane = 2/1) to give the product **24** (45.6 mg, 67.0 μmol, 82% in 2 steps from **22**) as a yellow solid.

*A procedure for the reaction of platform **24** with cyclooctyne **8***



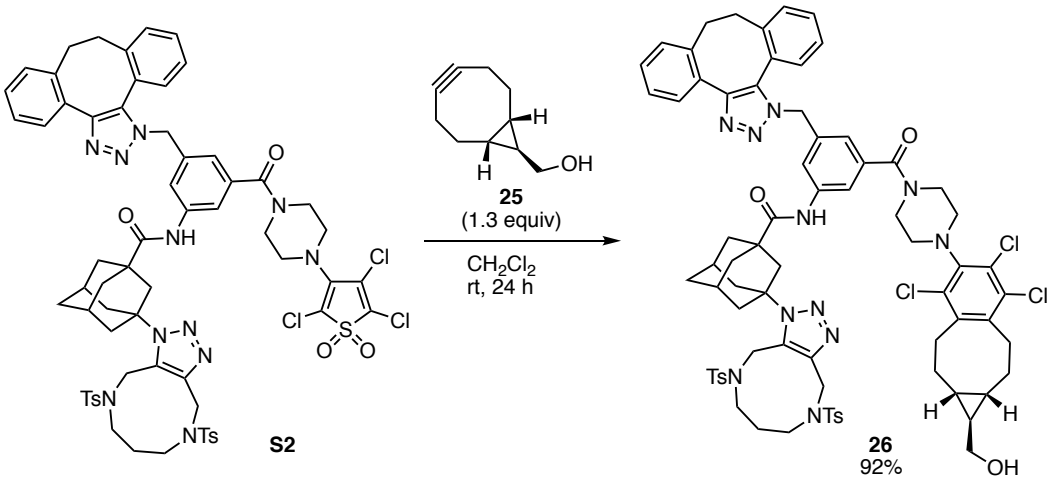
To a solution of platform molecule **24** (14.4 mg, 21.1 μmol) dissolved in CH_2Cl_2 (1.0 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a*,*e*]cyclooctene (**8**) (3.09 mg, 15.1 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (EtOAc/*n*-hexane = 3/1) to give platform–DBCO conjugate **S1** (12.6 mg, 14.2 μmol, 94%) as a yellow solid.

*A procedure for the reaction of platform–DBCO conjugate **S1** with cycloalkyne **14***



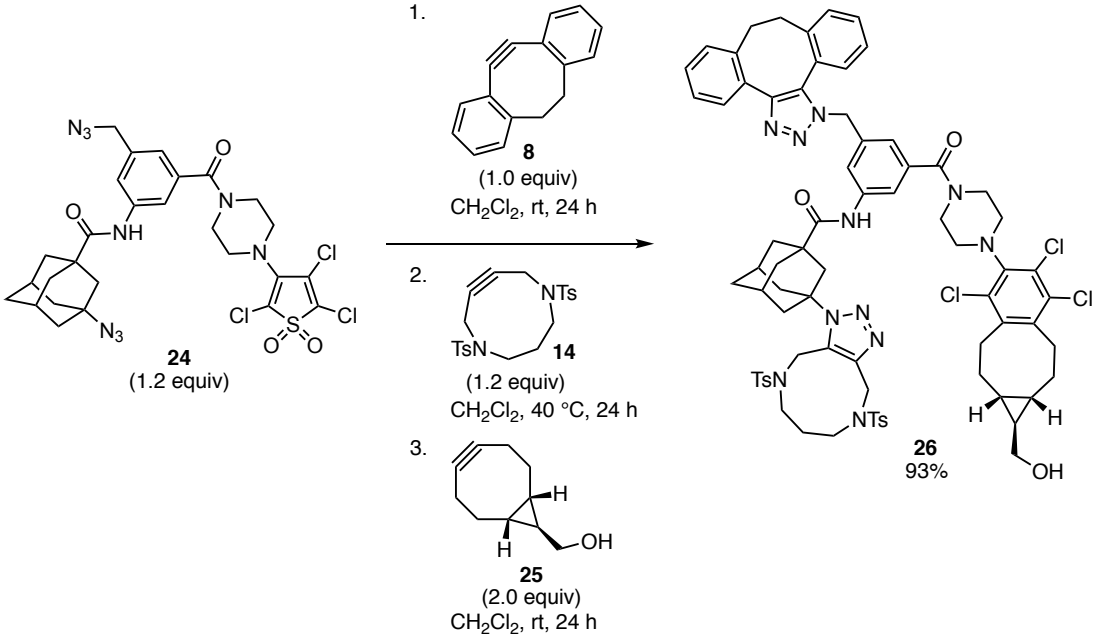
To a solution of platform–DBCO conjugate **S1** (9.25 mg, 10.4 μmol) dissolved in CH_2Cl_2 (0.20 mL) was added 4,8-ditosyl-4,8-diazacyclononyne (**14**) (4.33 mg, 10.0 μmol) at room temperature. The mixture was stirred at 40 ºC for 24 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (CH_2Cl_2/MeOH = 15/1) to give platform–DBCO–DACN conjugate **S2** (12.9 mg, 9.79 μmol, 98%) as a yellow solid.

*A procedure for the reaction of platform–DBCO–DACN conjugate **S2** with cycloalkyne **25***



To a solution of platform–DBCO–DACN conjugate **S2** (5.4 mg, 4.1 μmol) dissolved in CH_2Cl_2 (0.20 mL) was added (1*α*,8*α*,9*α*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**25**) (0.81 mg, 5.4 μmol) 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 (CH_2Cl_2/MeOH = 15/1) to give platform–DBCO–DACN–BCN conjugate **26** (5.3 mg, 3.8 μmol, 92%) as a colorless solid.

HPLC analysis of the sequential cycloaddition reaction in a one-pot manner



To a solution of platform molecule **24** (7.49 mg, 11.0 μmol) dissolved in CH_2Cl_2 (0.20 mL) was added 5,6-didehydro-11,12-dihydrodibenzo[*a*,*e*]cyclooctene (**8**) (2.01 mg, 9.84 μmol) at room temperature. After stirring for 24 h at the same temperature, a part of the solution (0.20 μL) was analyzed by HPLC (column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 60:40 (0–5 min), linear gradient from 60:40 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm) (Figure S1-B).

To the remaining reaction mixture was added 4,8-ditosyl-4,8-diazacyclononyne (**14**) (4.78 mg, 11.1 μmol) at room teperature. After stirring the mixture at 40 °C, a part of the solution (0.20 μL) was analyzed by HPLC (column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 60:40 (0–5 min), linear gradient from 60:40 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm) (Figure S1-C).

To the remaining reaction mixture was added (1*α*,8*α*,9*α*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**25**) (3.00 mg, 20.0 μmol) at room temperature. After stirring for 24 h at the same temperature, a part of the solution (0.20 μL) was analyzed by HPLC (column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 60:40 (0–5 min), linear gradient from 60:40 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm) (Figure S1-D). The remaining reaction mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (CH_2Cl_2/EtOAc/MeOH = 7/7/1) to give platform–DBCO–DACN–BCN conjugate **26** (12.9 mg, 9.19 μmol, 93% in 3 steps) as a colorless solid.

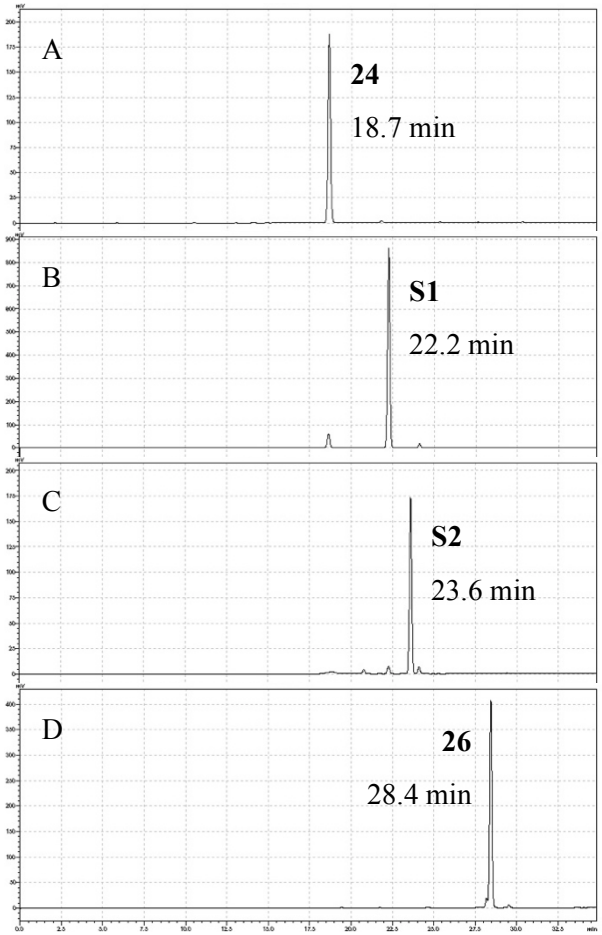
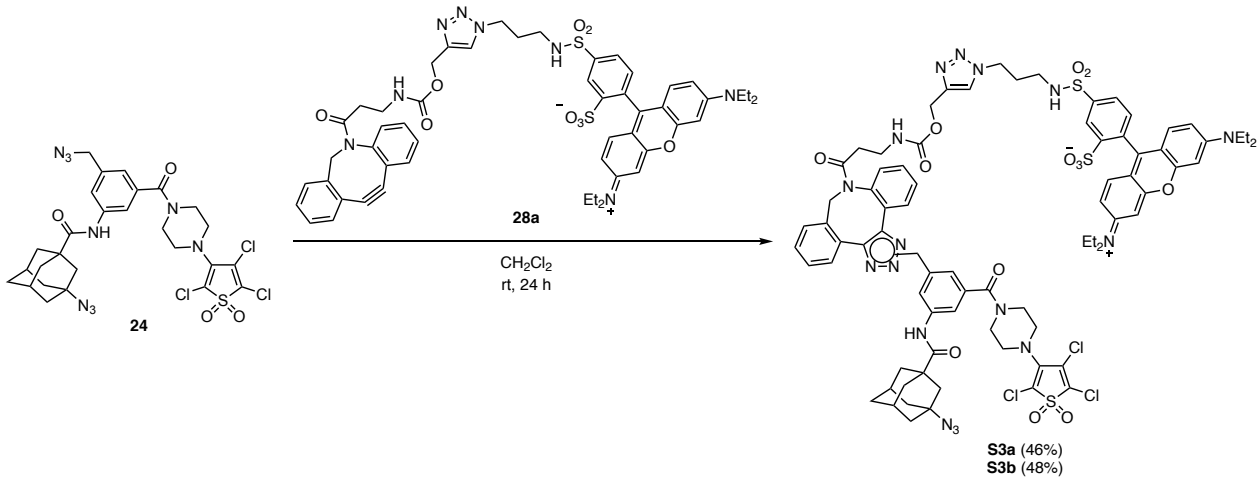


Figure S1. (A) HPLC chart of **24**. (B) HPLC chart for the crude reaction mixture of first step. (C) HPLC chart for the crude reaction mixture of second step. (D) HPLC chart for the crude reaction mixture of third step; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 60:40 (0–5 min), linear gradient from 60:40 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

*Preparation of authentic samples of platform-TESRA conjugates **S3a** and **S3b***



To a solution of platform molecule **24** (6.88 mg, 10.1 μmol) dissolved in CH_2Cl_2 (0.30 mL) was added a solution of 1-(2-(2-(2-(2-(4-(3,6-bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido) ethoxy)ethoxy)ethoxy)ethyl)-4-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropylaminocarbonyloxymethyl)-1*H*-[1,2,3]triazole (**28a**) (8.89 mg, 9.03 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. A part of the solution (0.20 μL) was analyzed by HPLC (Figure S2-A). The remaining residue was purified by flash column chromatography (silica-gel 4.0 g, CH_2Cl_2 only to CH_2Cl_2/EtOAc/MeOH = 7/7/1 to 5/5/1) to give platform–TESRA conjugate **S3a** (6.92 mg, 4.11 μmol, 46%) (Figure S2-B) as a purple solid and platform–TESRA conjugate **S3b** (7.29 mg, 4.33 μmol, 48%) as a purple solid (Figure S2-C).

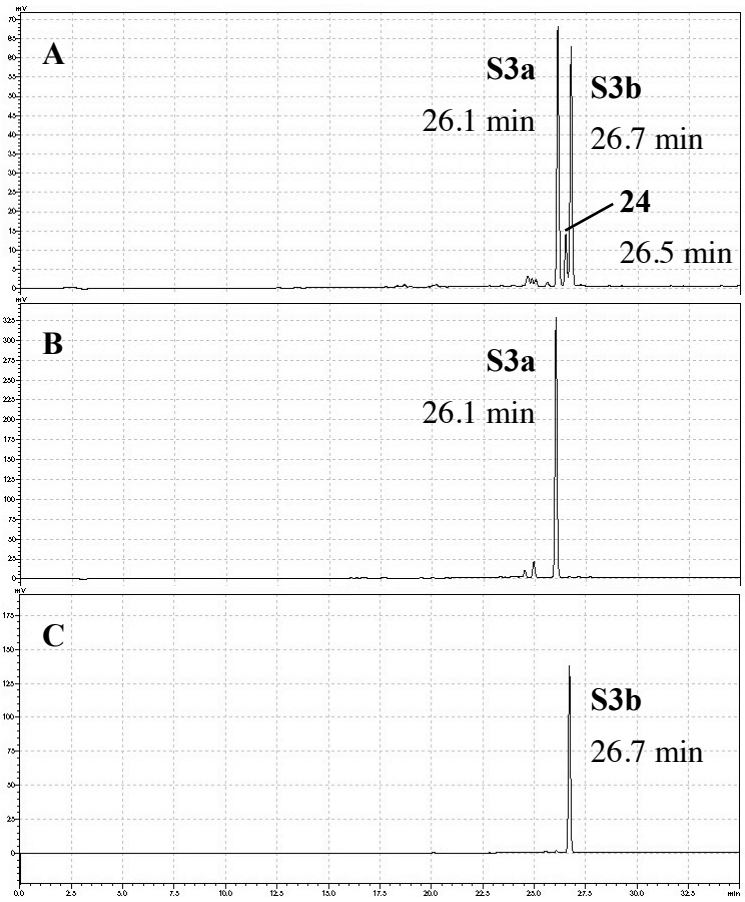
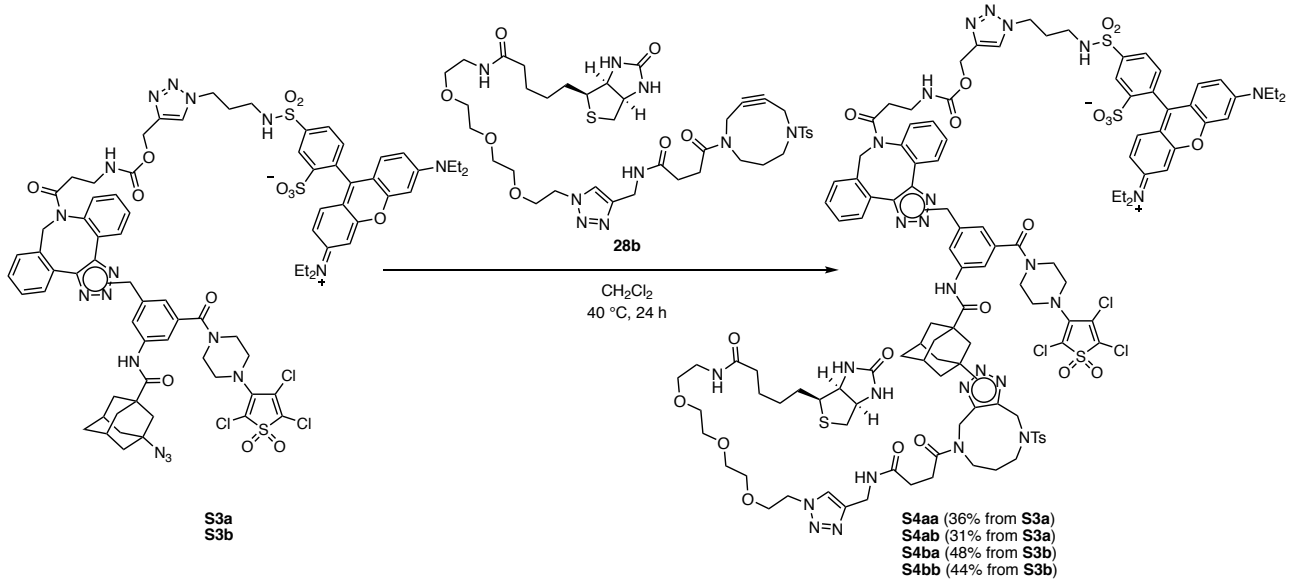


Figure S2. (A) HPLC chart for the crude reaction mixture of **24** with **28a**; three main peaks were observed at R*t* = 26.1 min (44%) for **S3a**, 26.5 min (9%) for **24** and 26.7 min (42%) for **S3b**. (B) HPLC chart of **S3a**. (C) HPLC chart of **S3b**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

*Preparation of authentic samples of platform–TESRA–biotin conjugates **S4aa**, **S4ab**, **S4ba**, and **S4bb***



To a solution of platform–TESRA conjugate **S3a** (3.40 mg, 2.02 μmol) dissolved in CH_2Cl_2 (0.30 mL) was added 1-(2-(2-(2-(2-(biotinamido)ethoxy)ethoxy)ethoxy)ethyl)-4-(3-(4-tosyl-4,8-diazacyclononyn-8-yl carbonyl)propionylaminomethyl)-1*H*-[1,2,3]triazole (**28b**) (1.57 mg, 1.83 μmol) at room temperature. After stirring the mixture at 40 ºC for 24 h, the mixture was concentrated under reduced pressure. A part of the solution (0.20 μL) was analyzed by HPLC (Figure S3-A). The remaining residue was purified by flash column chromatography (silica-gel 16 g, CH_2Cl_2 only to CH_2Cl_2/EtOAc/MeOH = 6/1/1 to 4/1/1 to 8/1/2) to give platform–TESRA–biotin conjugate **S4aa** (2.50 mg, ca. 66% purity determined by ^{1}H NMR analysis, ca. 0.650 μmol, ca. 36%) as a purple solid (Figure S3-B) and platform–TESRA–biotin conjugate **S4ab** (2.36 mg, ca. 62% purity determined by ^{1}H NMR analysis, ca. 0.576 μmol, ca. 31%) as a purple solid (Figure S3-C).

According to the procedure for the preparation of **S4aa** and **S4ab**, **S4ba** (1.78 mg, ca. 62% purity determined by ^{1}H NMR analysis, 0.434 μmol, ca. 48%) (Figure S4-B) and **S4bb** (1.52 mg, ca. 66% purity determined by ^{1}H NMR analysis, 0.395 μmol, ca. 44%) (Figure S4-C) were prepared using the corresponding platform–TESRA conjugate **S3b**.

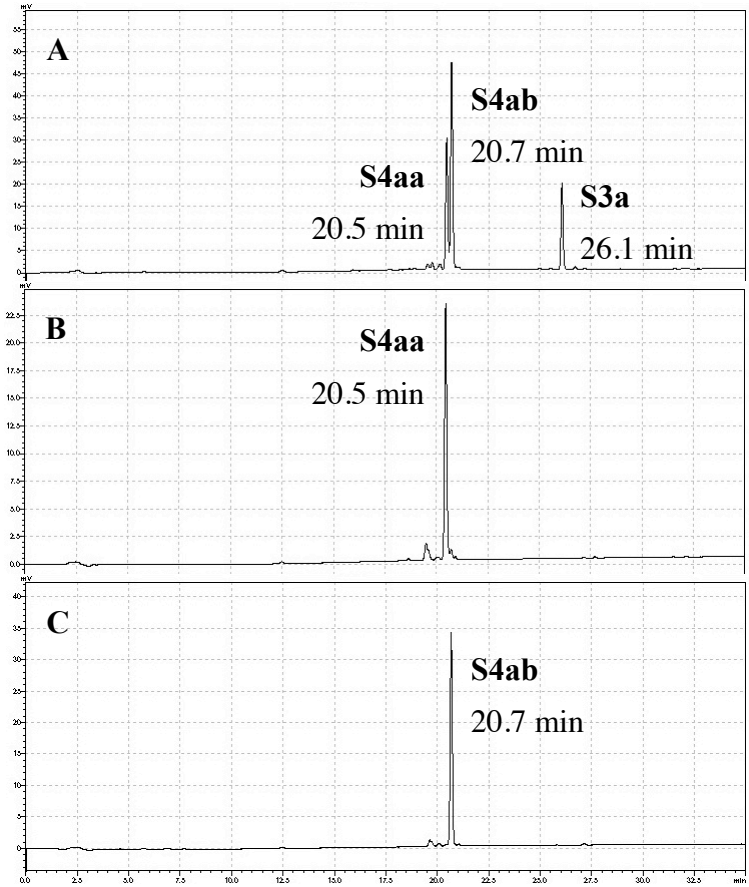


Figure S3. (A) HPLC chart for the crude reaction mixture of **S3a** with **28b**; three main peaks were observed at R*t* = 20.1 min (29%) for **S4aa**, 20.5 min (47%) for **S4ab** and 26.1 min (19%) for **S3a**. (B) HPLC chart of **S4aa**. (C) HPLC chart of **S4ab**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

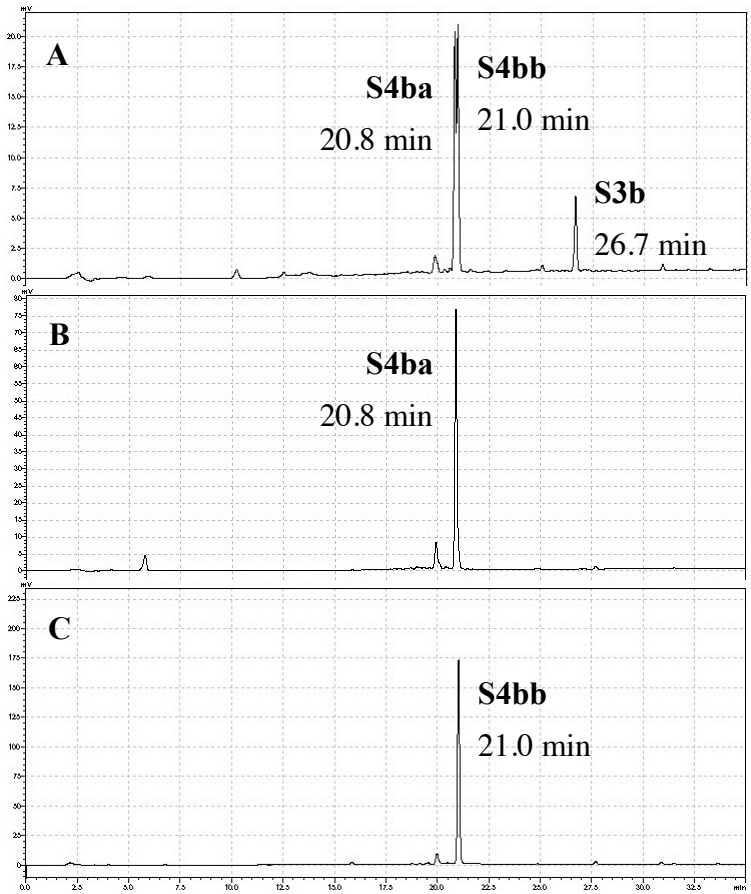
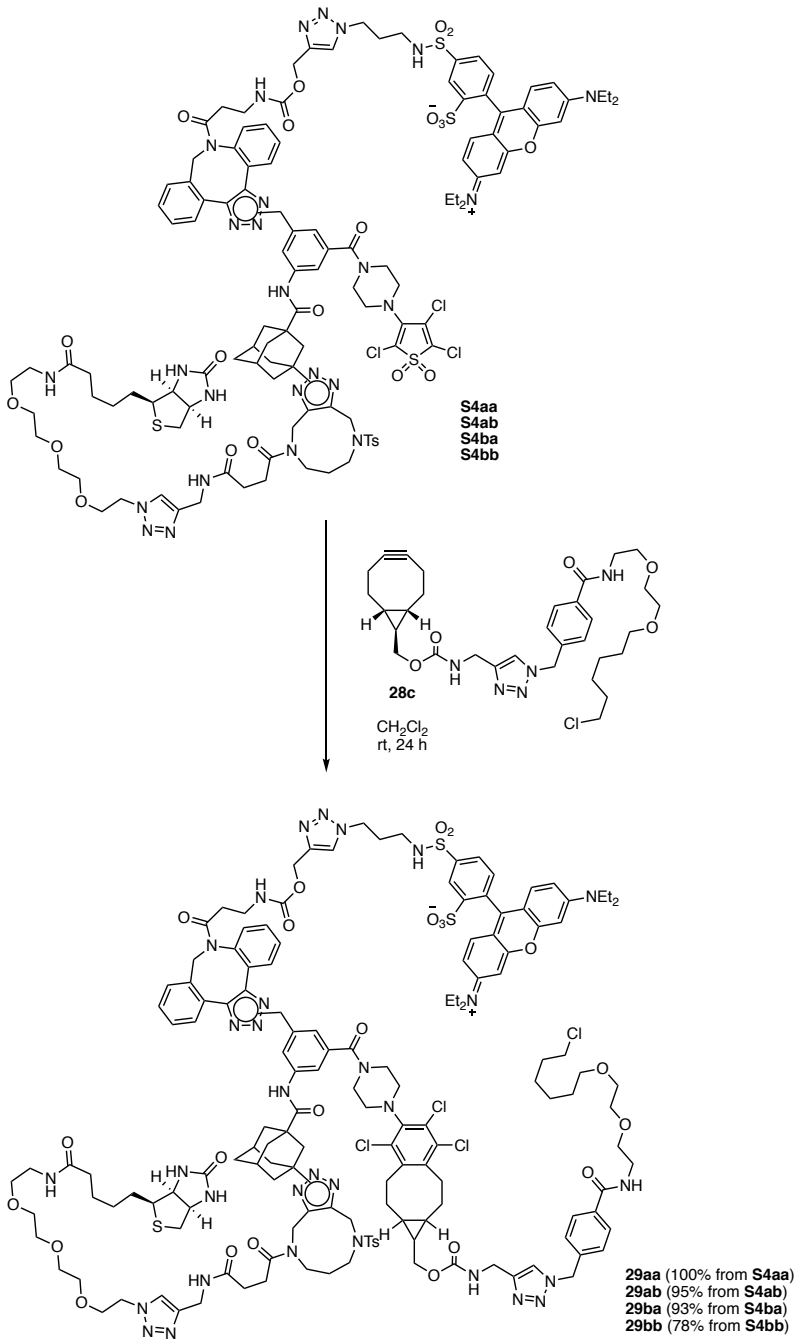


Figure S4. (A) HPLC chart for the crude reaction mixture of **S3b** with **28b**; three main peaks were observed at R*t* = 20.8 min (39%) for **S4ba**, 21.0 min (39%) for **S4bb** and 26.7 min (19%) for **S3b**. (B) HPLC chart of **S4ba**. (C) HPLC chart of **S4bb**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

*Preparation of authentic sample of platform–TESRA–biotin conjugates **29aa**, **29ab**, **29ba**, and **29bb***



To a solution of platform–TESRA–biotin **S4aa** (0.83 mg, ca. 66% purity determined by ^{1}H NMR analysis, ca. 0.22 μmol,) dissolved in CH_2Cl_2 (0.30 mL) was added 4-(((1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethoxycarbonyl)aminomethyl)-1-(4-(2-(2-(6-chlorohexoxy)ethoxy)ethylaminocarbonyl)benzyl)-1*H*-[1,2,3]triazole (**28c**) (0.33 mg, 0.54 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. A part of the solution (0.20 μL) was analyzed by HPLC (Figure S5-A). The remaining residue was purified by flash column chromatography (silica-gel 1.0 g, CH_2Cl_2 only to CH_2Cl_2/MeOH = 5/1) to give platform–TESRA–biotin–HaloTag ligand conjugate **29aa** (1.02 mg, ca. 67% purity determined by ^{1}H NMR analysis, ca. 0.22 μmol, ca. 100%). The product was determined by ^{1}H NMR, HPLC (Figure S5-B) and HRMS analyses.

According to the procedure for the preparation of **29aa**, **29ab** (1.48 mg, ca. 80% purity determined by ^{1}H NMR analysis, ca. 0.38 μmol, 95%) (Figure S6-B), **29ba** (1.70 mg ca. 69% purity determined by ^{1}H NMR analysis, 0.38 μmol, ca. 93%) (Figure S7-B), and **29bb** (1.62 mg, ca. 68% purity determined by ^{1}H NMR analysis, ca. 0.36 μmol, ca. 78%) (Figure S8-B) were prepared using the corresponding platform–TESRA–biotin conjugates **S4ab**, **S4ba**, and **S4bb**.

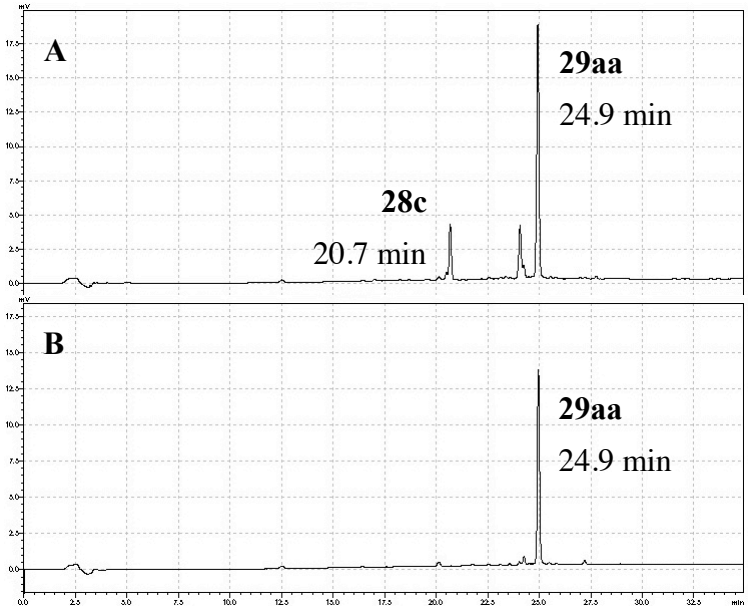


Figure S5. (A) HPLC chart for the crude reaction mixture of **S4aa** with **28c**; two peaks were observed at R*t* = 20.7 min (15%) for **28c** and 24.9 min (70%) for **29aa**. (B) HPLC chart of **29aa**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

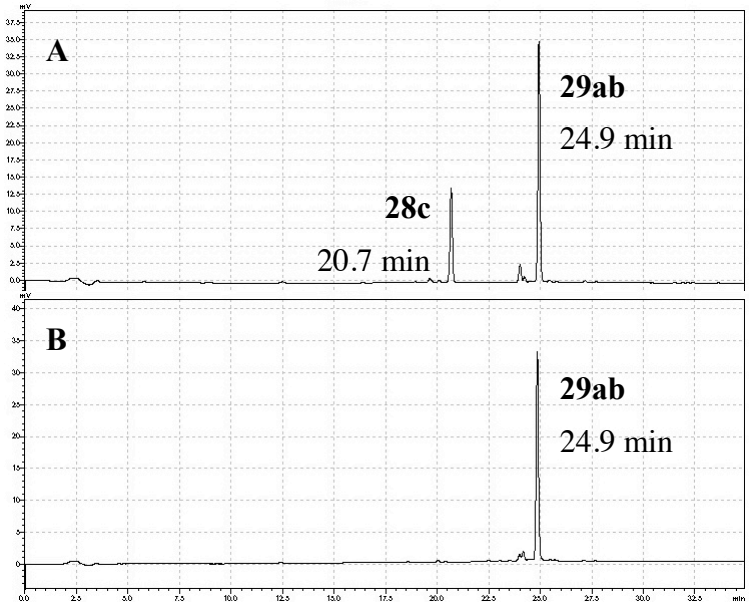


Figure S6. (A) HPLC chart for the crude reaction mixture of **S4ab** with **28c**; two main peaks were observed at R*t* = 20.7 min (29%) for **28c** and 24.9 min (66%) for **29ab**. (B) HPLC chart of **29ab**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

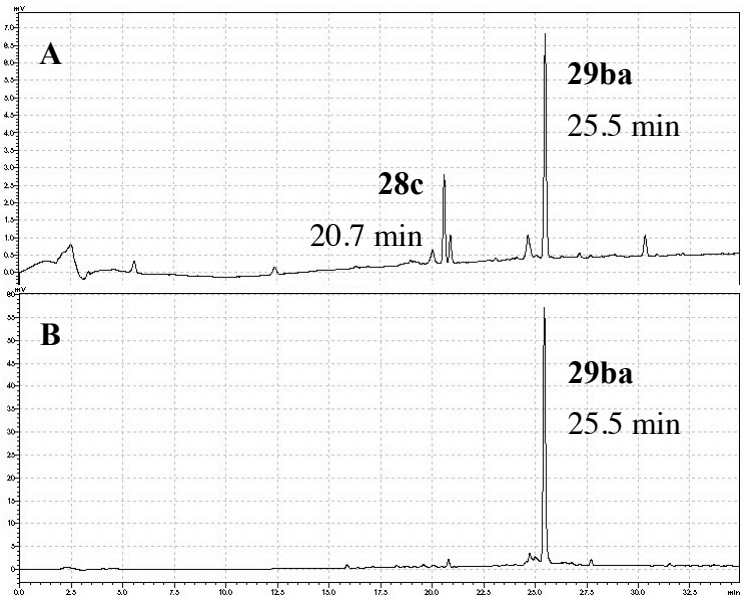


Figure S7. (A) HPLC chart for the crude reaction mixture of **S4ba** with **28c**; two main peaks were observed at R*t* = 20.7 min (20%) for **28c** and 25.5 min (55%) for **29ba**. (B) HPLC chart of **29ba**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

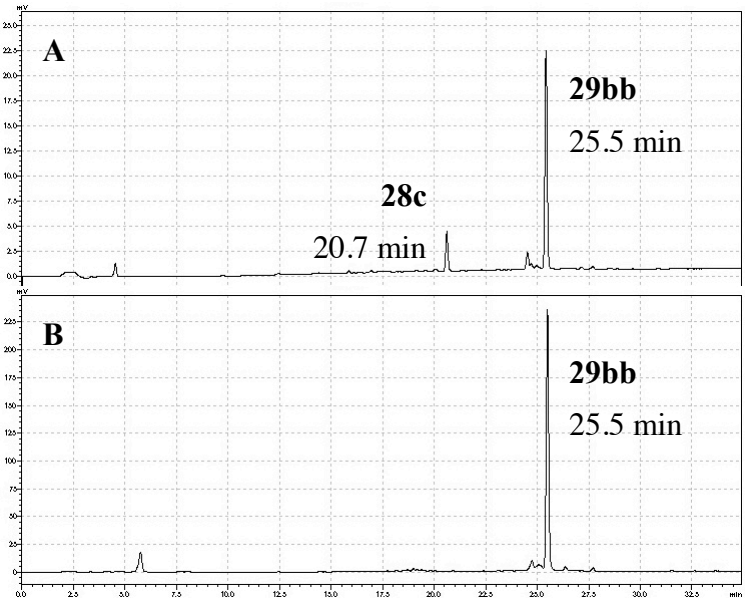
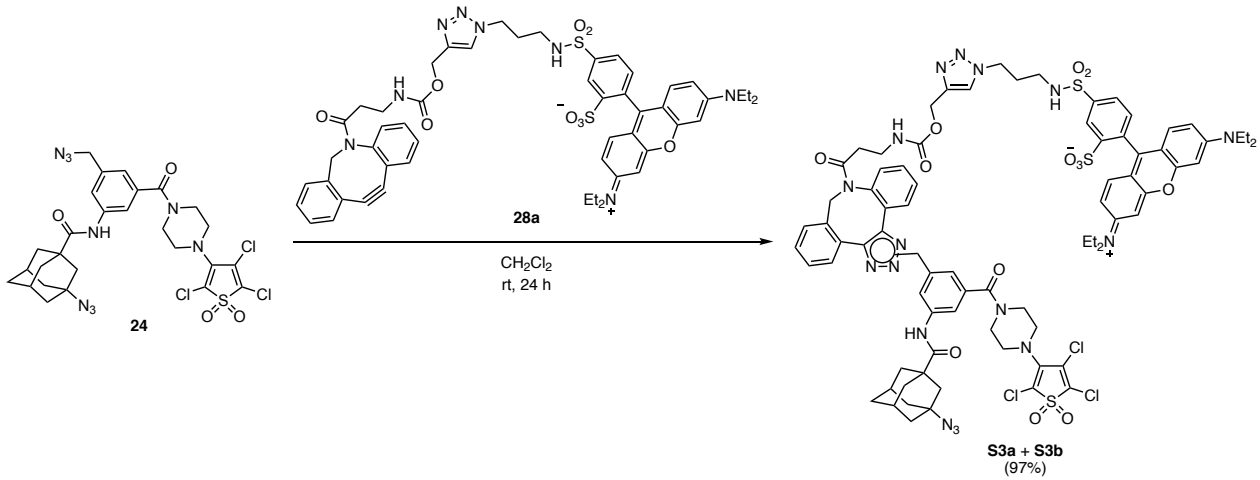


Figure S8. (A) HPLC chart for the crude reaction mixture of **S4bb** with **28c**; two main peaks were observed at R*t* = 20.7 min (14%) for **28c** and 25.5 min (79%) for **29bb**. (B) HPLC chart of **29bb**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

*A procedure for the reaction of platform **24** with cyclooctyne **28a***



To a solution of platform molecule **24** (1.37 mg, 2.01 μmol) dissolved in CH_2Cl_2 (0.30 mL) was added a solution of 1-(2-(2-(2-(2-(4-(3,6-bis(diethylamino)xanthylium-9-yl)-3-sulfonatobenzenesulfonamido)ethoxy) ethoxy)ethoxy)ethyl)-4-(3-(5*H*,6*H*-11,12-didehydrodibenzo[*b*,*f*]azocin-5-yl)-3-oxopropylaminocarbonyloxymethyl)-1*H*-[1,2,3]triazole (**28a**) (1.87 mg, 1.90 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. A part of the solution (0.20 μL) was analyzed by HPLC (Figure S9-A). The remaining residue was purified by flash column chromatography (silica-gel 5 g, CH_2Cl_2 only to CH_2Cl_2/EtOAc/MeOH = 5/4/1 to CH_2Cl_2/MeOH = 8/1) to give platform–TESRA conjugates **S3a** + **S3b** (3.99 mg, ca. 78% purity determined by ^{1}H NMR analysis, ca. 1.84 μmol, ca. 97%) as a purple solid (Figure S9-B).

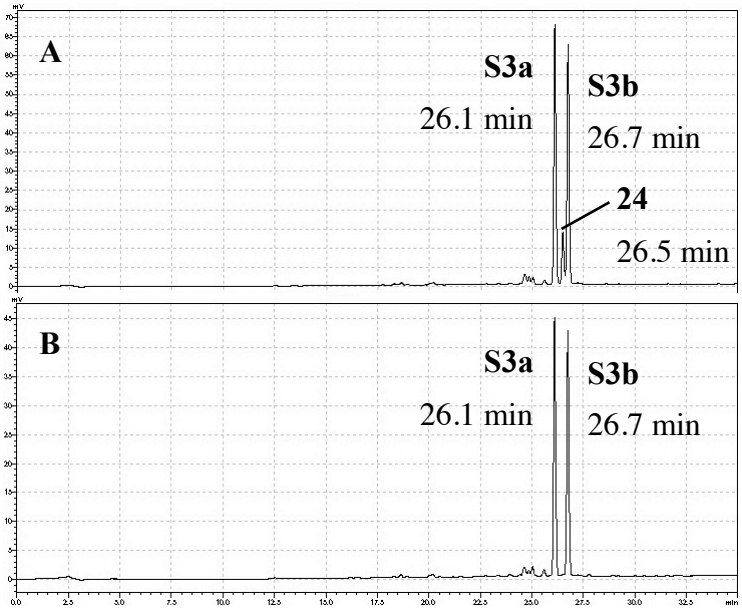
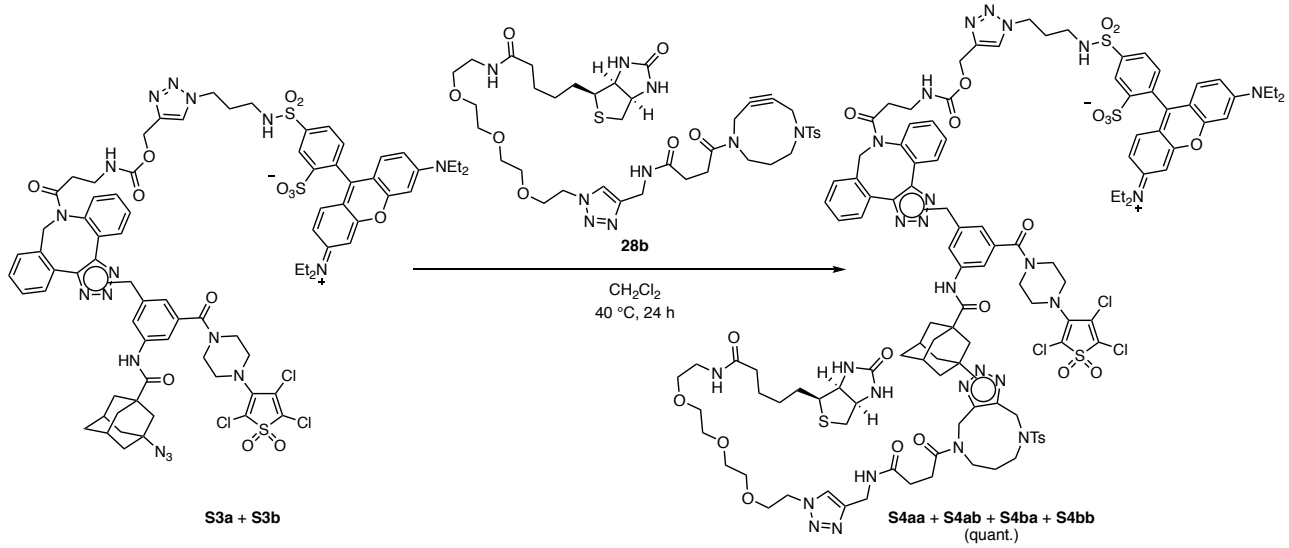


Figure S9. (A) HPLC chart for the crude reaction mixture of **24** with **28a**; three main peaks were observed at R*t* = 26.1 min (44%) for **S3a**, 26.5 min (9%) for **24** and 26.7 min (42%) for **S3b**. (B) HPLC chart of **S3a** + **S3b**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

*A procedure for the reaction of platform–TESRA conjugates **S3a** and **S3b** with cycloalkyne **28b***



To a solution of platform–TESRA conjugate **S3a** + **S3b** (3.32 mg, ca. 78% purity determined by ^{1}H NMR analysis, ca. 1.54 μmol) dissolved in CH_2Cl_2 (0.30 mL) was added 1-(2-(2-(2-(2-(biotinamido)ethoxy) ethoxy)ethoxy)ethyl)-4-(3-(4-tosyl-4,8-diazacyclononyn-8-ylcarbonyl)propionylaminomethyl)-1*H*-[1,2,3]triazole (**28b**) (1.23 mg, 1.43 μmol) at room temperature. After stirring the mixture at 40 ºC for 24 h, the mixture was concentrated under reduced pressure. A part of the solution (0.20 μL) was analyzed by HPLC (Figure S10-A). The remaining residue was purified by flash column chromatography (silica-gel 4.0 g, CH_2Cl_2 only to CH_2Cl_2/MeOH = 9/1 to 4/1) to give platform–TESRA–biotin conjugate **S4aa** + **S4ab** + **S4ba** + **S4bb** (4.95 mg, ca. 76% purity determined by ^{1}H NMR analysis, ca. 1.48 μmol, quant.) as a purple solid (Figure S10-B).

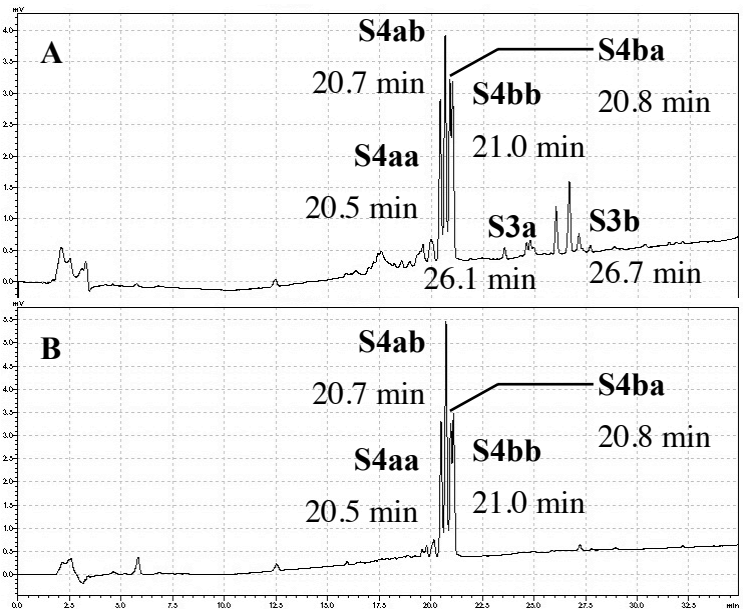
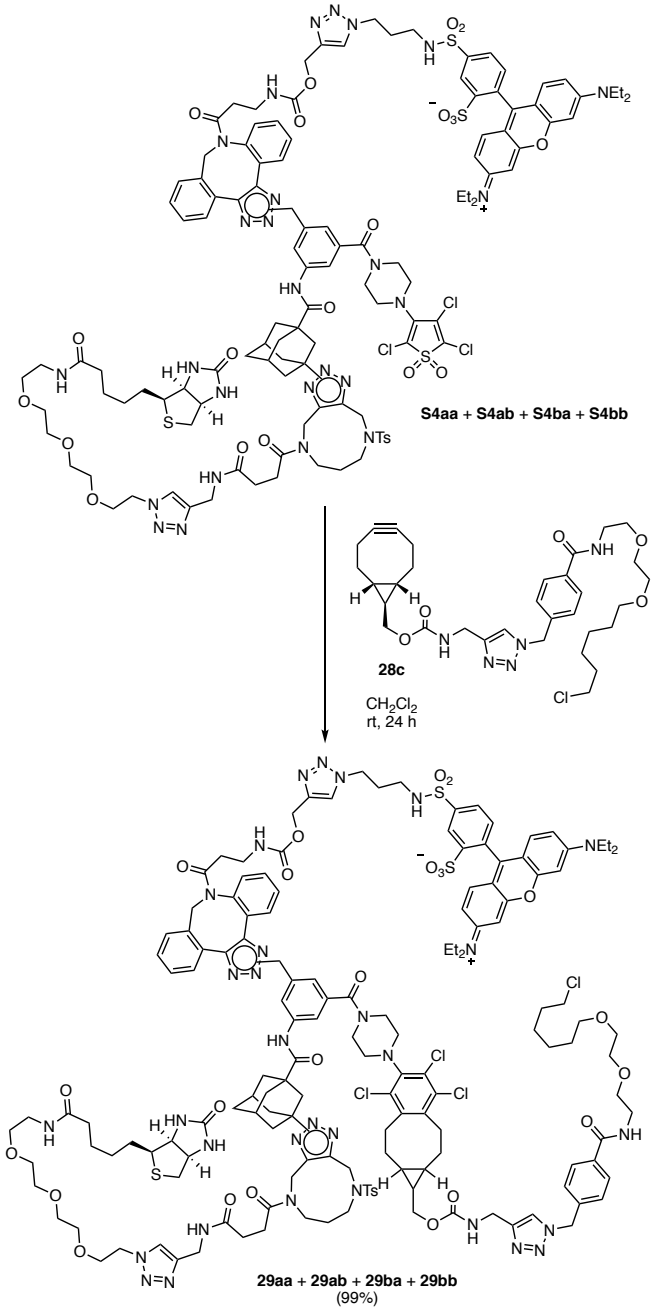


Figure S10. (A) HPLC chart for the crude reaction mixture of **S3** with **28b**; six main peaks were observed at R*t* = 20.5 min (15%) for **S4aa**, 20.7 min (21%) for **S4ab**, 20.8 min (16%) for **S4ba,** 21.0 min (16%) for **S4bb**, 26.1 min (4%) for **S3a**, and 26.7 min (7%) for **S3b.** (B) HPLC chart of **S4**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

*A procedure for reaction of platform–TESRA–biotin conjugates **S4aa**, **S4ab**, **S4ba**, and **S4bb** with cycloalkyne **28c***



To a solution of platform–TESRA–biotin conjugates **S4aa** + **S4ab** + **S4ba** + **S4bb** (3.35 mg, ca. 76% purity determined by ^{1}H NMR analysis, ca 1.00 μmol) dissolved in CH_2Cl_2 (0.30 mL) was added 4-((1α,8α,9α)-bicyclo[6.1.0]non-4-yn-9-ylmethoxycarbonylaminomethyl)-1-(4-(2-(2-(6-chlorohexoxy)ethoxy)ethylaminocarbonyl)benzyl)-1*H*-[1,2,3]triazole (**28c**) (1.13 mg, 1.84 μmol) at room temperature. After stirring for 24 h at the same temperature, the mixture was concentrated under reduced pressure. A part of the solution (0.20 μL) was analyzed by HPLC (Figure S11-A). The remaining residue was purified by flash column chromatography (silica-gel 2.0 g, CH_2Cl_2/MeOH = 10/1 to 5/1) to give platform–TESRA–biotin–HaloTag-ligand conjugate **29aa** + **29ab** + **29ba** + **29bb** (4.24 mg, ca. 72% purity determined by ^{1}H NMR analysis, ca. 0.988 μmol, 99%) as a purple solid (Figure S11-B).

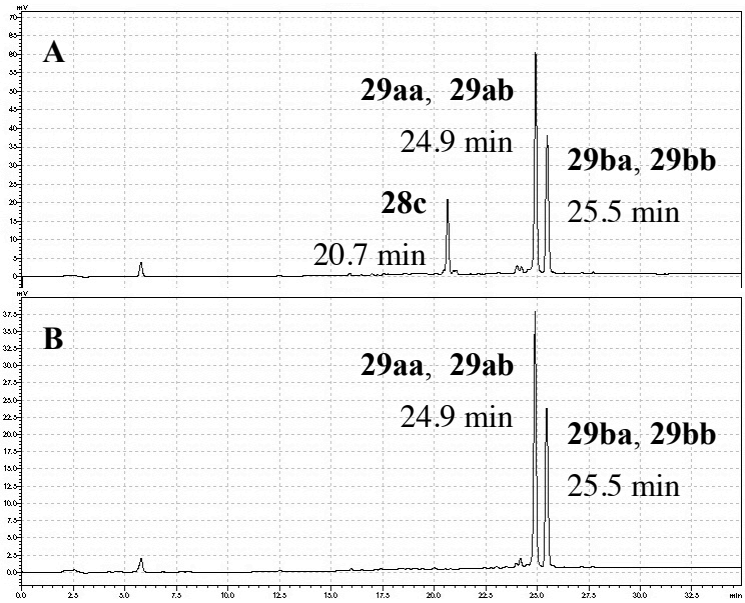
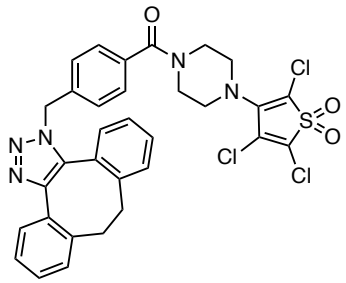


Figure S11. (A) HPLC chart for the crude reaction mixture of **S4** with **28c**; three main peaks were observed at R*t* = 20.7 min (14%) for **28c**, 24.9 min (46%) for **29aa** and **29ab**, 25.5 min (32%) for **29ba** and **29bb.** (B) HPLC chart of **29**; column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm.

Characterization Data of New Compounds

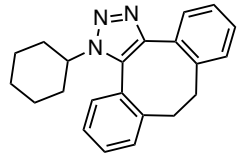
1-Benzyl-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**11a**)[S11] was identical in spectra data with those reported in the literature.

3-(4-(4-(8,9-Dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazol-1-yl)methyl)benzoyl)piperazin-1-yl)-2,4,5-trichlorothiophene *S*,*S*-dioxide (**9**)



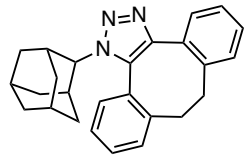
Yellow solid; Mp 105 °C (decomp.); TLC R_f 0.37 (*n*-hexane/EtOAc = 1/3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.75–2.80 (m, 1H), 2.89–2.95 (m, 1H), 3.03–3.10 (m, 1H), 3.32–3.44 (m, 7H), 3.68–4.02 (br, 2H), 5.57 (d, 1H, *J* = 15.1 Hz), 5.62 (d, 1H, *J* = 15.1 Hz), 7.09 (d, 1H, *J* = 7.6 Hz), 7.14–7.24 (m, 6H), 7.28–7.35 (m, 4H), 7.53–7.55 (m, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 32.7, 36.4, 41.6–42.6 (br), 47.3–48.1 (br), 49.8–50.5 (br, two signals overlapped), 51.6, 106.9, 126.0 (two signals overlapped), 126.4, 127.6, 127.7, 128.1, 128.8, 129.5, 129.9, 130.0, 130.1, 130.9, 131.7, 131.9, 134.0, 134.6, 137.5, 137.7, 141.3, 141.5, 147.0, 169.9; IR (KBr, cm^{-1}) 750, 986, 1152, 1261, 1431, 1566, 1638, 2930; HRMS (ESI^+) *m*/*z* 688.0731 ($[M+Na]^+$, $C_{32}H_{26}{}^{35}Cl_3N_5NaO_3S^+$ requires 688.0714).

1-Cyclohexyl-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**11b**)



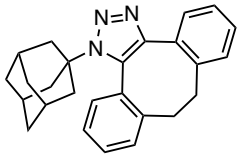
Colorless solid; Mp 180–183 °C; TLC R_f 0.57 (*n*-hexane/EtOAc = 1/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.17–1.25 (m, 1H), 1.29–1.44 (m, 2H), 1.69–1.82 (m, 3H), 1.98–2.06 (m, 2H), 2.29–2.42 (m, 2H), 2.86–2.94 (m, 1H), 3.07–3.14 (m, 2H), 3.37–3.45 (m, 1H), 4.17–4.23 (m, 1H), 7.11–7.20 (m, 4H), 7.26–7.28 (m, 1H), 7.33–7.39 (m, 2H), 7.50–7.51 (m, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 24.9, 25.3, 25.5, 32.8, 32.9, 33.9, 36.6, 58.0, 125.9, 126.4, 127.0, 127.8, 128.8, 129.5, 130.0, 130.1, 130.8, 131.8, 132.9, 137.5, 141.7, 146.2; IR (KBr, cm^{-1}) 764, 984, 1169, 1263, 1340, 1452, 1502, 2934; HRMS (ESI^+) *m*/*z* 352.1793 ($[M+Na]^+$, $C_{22}H_{23}N_3Na^+$ requires 352.1784).

1-(Adamantan-2-yl)-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**11c**)



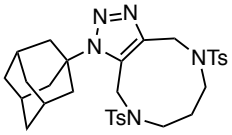
Colorless solid; Mp 210–212 °C; TLC R_f 0.66 (*n*-hexane/EtOAc = 1/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.51–1.54 (m, 1H), 1.58–1.61 (m, 1H), 1.73–1.83 (m, 5H), 1.88–1.91 (m, 2H), 1.95–2.01 (br, 1H), 2.05–2.08 (m, 1H), 2.16–2.20 (m, 1H), 2.71–2.78 (br, 1H), 2.84–2.93 (m, 1H), 2.96–2.99 (m, 1H), 3.06–3.13 (m, 2H), 3.35–3.43 (m, 1H), 4.66 (s, 1H), 7.12–7.20 (m, 4H), 7.23 (ddd, 1H, *J* = 7.4, 7.4, 1.5 Hz), 7.29–7.35 (m, 2H), 7.50–7.52 (m, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 27.0, 27.3, 30.8, 31.9, 32.1, 32.9, 33.0, 36.5, 37.6, 37.7, 38.3, 63.3, 125.9, 126.3, 127.8 (two signals overlapped), 128.1, 129.3, 130.0, 130.2, 130.7, 131.7, 133.4, 137.6, 141.1, 146.4; IR (KBr, cm^{-1}) 761, 966, 1024, 1157, 1263, 1332, 1452, 1502, 2907; HRMS (ESI^+) *m*/*z* 382.2275 ($[M+H]^+$, $C_{26}H_{28}N_3{}^+$ requires 382.2278).

1-(Adamantan-1-yl)-8,9-dihydro-1*H*-dibenzo[3,4:7,8]cycloocta[1,2-*d*][1,2,3]triazole (**11d**)



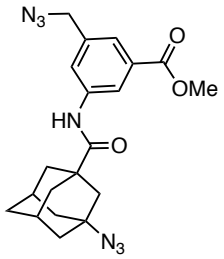
Colorless solid; Mp 211–213 °C; TLC R_f 0.55 (*n*-hexane/EtOAc = 1/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.64–1.71 (m, 6H), 2.12–2.15 (br, 3H), 2.24–2.26 (m, 3H), 2.35–2.37 (m, 3H), 2.83–3.00 (m, 3H), 3.24–3.29 (m, 1H), 7.03–7.05 (m, 1H), 7.11–7.17 (m, 3H), 7.24–7.28 (m, 3H), 7.40–7.42 (m, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 29.7, 32.7, 35.8, 36.3, 42.9, 63.0, 125.5, 125.9, 128.0, 129.1, 129.4, 129.8, 130.3, 130.6, 130.8, 131.2, 133.5, 138.0, 141.0, 148.2; IR (KBr, cm^{-1}) 735, 1011, 1101, 1125, 1263, 1308, 1325, 1358, 1435, 1452, 1479, 2853, 2909; HRMS (ESI$^+$) *m*/*z* 404.2098 ([M+Na]$^+$, $C_{26}H_{27}N_3Na^+$ requires 404.2097).

1-(Adamantan-1-yl)-4,5,6,7,9,10-hexahydro-4,8-ditosyl-4,8-diazacyclonona[*d*][1,2,3]triazole (**15**)



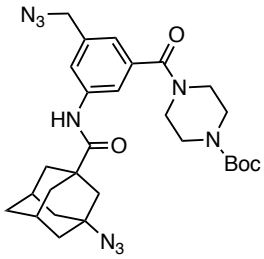
Colorless solid; Mp 245 °C (decomp.); TLC R_f 0.24 (*n*-hexane/EtOAc = 1/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.66–1.70 (m, 2H), 1.77–1.83 (m, 6H), 2.25–2.30 (br, 3H), 2.39–2.40 (m, 6H), 2.45 (s, 3H), 2.47 (s, 3H), 2.84–2.87 (m, 2H), 3.09–3.11 (m, 2H), 4.35 (s, 2H), 4.92 (s, 2H), 7.33–7.34 (AA'BB', 2H), 7.41–7.42 (AA'BB', 2H), 7.66–7.68 (AA'BB', 2H), 7.79–7.80 (AA'BB', 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 21.5, 21.6, 29.6, 30.2, 35.7, 41.4, 41.6, 45.6, 47.5, 49.1, 63.8, 127.2, 127.5, 128.1, 129.8, 130.1, 133.9, 135.9, 143.75, 143.85, 144.1; IR (KBr, cm^{-1}) 652, 685, 700, 712, 739, 816, 1090, 1161, 1306, 1340, 1452, 2853, 2912; HRMS (ESI$^+$) *m*/*z* 632.2320 ([M+Na]$^+$, $C_{31}H_{39}N_5NaO_4S_2^+$ requires 632.2336).

1-(3-Azidoadamantane-1-carboxamido)-3-(azidomethyl)-5-(methoxycarbonyl)benzene (**20**)



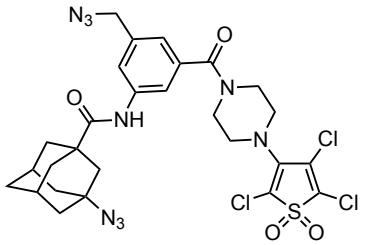
Colorless oil; TLC R_f 0.77 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.67–1.73 (br, 2H), 1.81–1.87 (m, 4H), 1.90–1.95 (br, 4H), 1.96–2.04 (br, 2H), 2.35–2.42 (br, 2H), 3.93 (s, 3H), 4.40 (s, 2H), 7.44 (s, 1H), 7.75 (s, 1H) 7.97 (s, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 29.5, 34.7, 37.9, 40.4, 43.0, 44.0, 52.4, 54.2, 58.8, 120.6, 123.9, 124.8, 131.4, 137.1, 138.4, 166.2, 174.4; IR (KBr, cm^{-1}) 1220, 1425, 1537, 1543, 1657, 1724, 2091, 2925; HRMS (ESI$^+$) *m*/*z* 432.1752 ([M+Na]$^+$, $C_{20}H_{23}N_7NaO_3^+$ requires 432.1755).

1-(3-Azidoadamantane-1-carboxamido)-3-azidomethyl-5-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)carbonylbenzene (**22**)



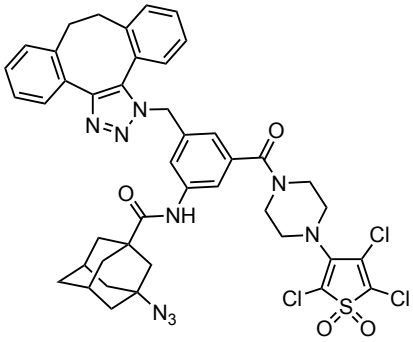
Colorless amorphous solid; Mp 91–93 °C; TLC R_f 0.55 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.47 (s, 9H), 1.65–1.72 (br, 4H), 1.80–1.87 (m, 4H), 1.88–1.92 (br, 2H), 1.97–2.01 (br, 2H), 2.34–2.40 (br, 2H), 3.33–3.59 (br, 6H), 3.67–3.81 (br, 2H), 4.36 (s, 2H), 7.08 (s, 1H), 7.58 (s, 1H), 7.61 (s, 1H), 7.69 (br s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 28.3, 29.5, 34.7, 37.8, 40.4, 42.0–42.3 (br), 42.9, 44.0, 47.4–47.7 (br), 54.1, 58.9, 80.4, 118.6, 120.9, 121.9, 136.6, 137.1, 138.6, 154.5, 169.6, 174.5; IR (KBr, cm^{-1}) 1167, 1239, 1419, 1689, 2090; HRMS (ESI$^+$) *m*/*z* 586.2846 ([M+Na]$^+$, $C_{28}H_{37}N_9NaO_4^+$ requires 586.2861).

2,3,5-Trichloro-4-(4-(1-(3-azidoadamantane-1-carboxamido)-3-azidomethylbenzene-5-carbonyl)piperazin-1-yl)thiophene *S*,*S*-dioxide (**24**)



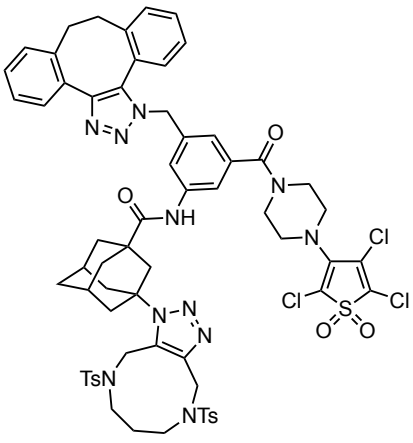
Yellow solid; Mp 167 °C (decomp.); TLC R_f 0.38 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.67–1.73 (br, 2H), 1.81–1.88 (m, 4H), 1.89–1.93 (br, 4H), 1.97–2.01 (m, 2H), 2.34–2.42 (br, 2H), 3.28–4.00 (br, 8H), 4.39 (s, 2H), 7.13 (s, 1H), 7.48 (s, 1H), 7.51 (s, 1H), 7.71 (s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 29.5, 34.7, 37.9, 40.4, 42.0–42.6 (br), 43.0, 44.0, 47.5–48.0 (br), 49.8–50.5 (br, two signals overlapped), 54.1, 58.8, 107.0, 118.7, 120.9, 122.3, 130.1, 132.0, 136.1, 137.4, 138.5, 141.4, 169.5, 174.5; IR (KBr, cm^{-1}) 1151, 1169, 1239, 1326, 1419, 1437, 1451, 1543, 1564, 1610, 2091; HRMS (ESI^+) *m*/*z* 680.1094 ($[M+H]^+$, $C_{27}H_{29}{}^{35}Cl_3N_9O_4S^+$ requires 680.1123).

Platform–DBCO conjugate **S1**



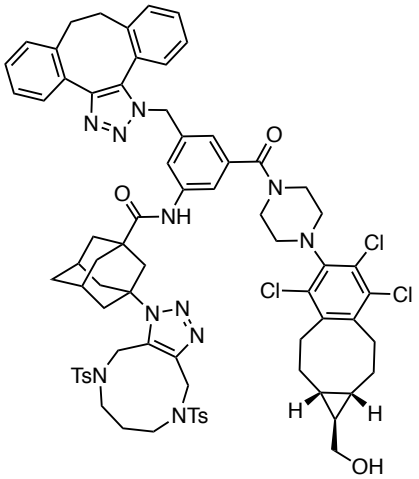
Yellow solid; Mp >300 °C; TLC R_f 0.38 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.64–1.73 (br, 2H), 1.79–1.88 (m, 8H), 1.93–1.98 (br, 2H), 2.33–2.41 (br, 2H), 2.80–2.84 (m, 1H), 2.93–2.98 (m, 1H), 3.04–3.11 (m, 1H), 3.32–3.52 (m, 7H), 3.72–3.93 (br, 2H), 5.51 (d, 1H, *J* = 15.3 Hz), 5.59 (d, 1H, *J* = 15.3 Hz), 6.88 (s, 1H), 7.08 (d, 1H, *J* = 7.6 Hz), 7.15–7.26 (m, 5H), 7.30–7.36 (m, 2H), 7.47–7.51 (m, 2H), 7.81 (s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 29.5, 32.9, 34.7, 36.4, 37.9, 40.4, 42.1–42.8 (br), 43.0, 44.0, 47.5–48.0 (br), 49.7–50.6 (br, two signals overlapped), 51.5, 58.8, 106.9, 119.0, 120.3, 121.8, 125.9, 126.1, 126.6, 128.3, 129.0, 129.4, 130.0, 130.1, 130.3, 131.0, 131.6, 132.0, 134.3, 136.1, 137.1, 137.6, 138.5, 141.4, 141.6, 147.1, 169.2, 174.4; IR (KBr, cm^{-1}) 1151, 1169, 1328, 1453, 1611, 2090; HRMS (ESI^+) *m*/*z* 884.2068 ($[M+H]^+$, $C_{43}H_{41}{}^{35}Cl_3N_9O_4S^+$ requires 884.2062).

Platform–DBCO–DACN conjugate **S2**



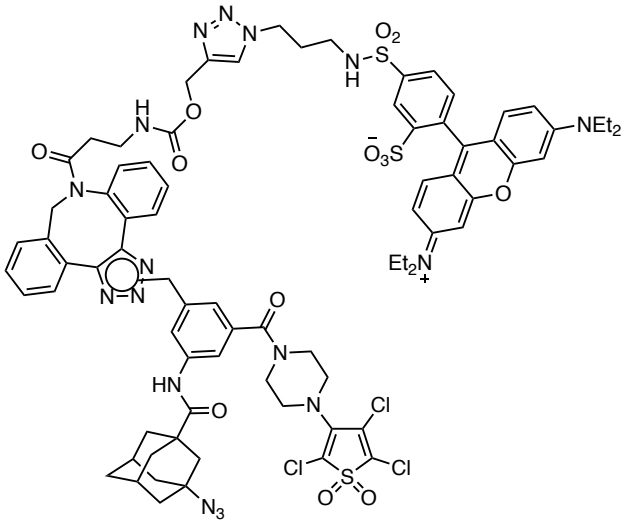
Yellow solid; Mp 126 °C (decomp.); TLC R_f 0.22 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.47–1.55 (br, 2H), 1.76–1.83 (m, 2H), 2.00–2.02 (m, 2H), 2.08–2.11 (m, 2H), 2.38–2.43 (m, 8H), 2.48–2.52 (m, 2H), 2.56–2.64 (br, 4H), 2.65–2.72 (br, 2H), 2.77–2.84 (m, 1H), 2.96–3.08 (m, 4H), 3.27–3.50 (m, 7H), 3.67–3.89 (br, 2H), 4.34 (s, 2H), 5.05 (s, 2H), 5.51 (d, 1H, J = 15.2 Hz), 5.56 (d, 1H, J = 15.2 Hz), 6.86 (s, 1H), 7.09 (d, 1H, J = 7.6 Hz), 7.12–7.14 (m, 1H), 7.16–7.20 (m, 3H), 7.26–7.27 (m, 2H), 7.32–7.33 (AA'BB', 2H), 7.35–7.36 (AA'BB', 2H), 7.50–7.52 (m, 1H), 7.55 (s, 1H), 7.60–7.62 (AA'BB', 2H), 7.74–7.76 (m, 3H), 8.35 (s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 21.5, 21.7, 29.4, 29.6, 32.9, 34.7, 36.4, 37.5, 40.5, 40.8–40.9 (br), 41.4, 43.9, 45.4, 47.6–47.7 (br, two signals overlapped), 47.8, 49.4, 49.8–50.6 (br, two signals overlapped), 51.6, 64.3, 106.6, 119.0, 120.9, 121.5, 125.9, 126.0, 126.5, 127.1, 127.4, 127.7, 128.2, 129.0, 129.7, 129.8, 129.9, 130.0, 130.1, 130.5, 130.8, 131.7, 132.0, 133.4, 134.3, 135.1, 135.6, 137.1, 137.7, 139.0, 141.4, 141.5, 143.9, 144.2, 144.9, 146.8, 169.5, 174.5; IR (KBr, cm^{-1}) 550, 579, 737, 986, 1090, 1159, 1234, 1306, 1331, 1435, 1452, 1545, 1609, 1641; HRMS (ESI$^+$) *m*/*z* 1338.3038 ([M+Na]$^+$, $C_{64}H_{64}{}^{35}Cl_3N_{11}NaO_8S_3^+$ requires 1338.3059).

Platform–DBCO–DACN–BCN conjugate **26**



Colorless solid; Mp 160 °C (decomp.); TLC R_f 0.56 (CH_2Cl_2/MeOH = 9/1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.48–0.63 (br, 2H), 0.73–0.78 (m, 1H), 1.30–1.42 (br, 2H), 1.43–1.52 (m, 2H), 1.76–1.83 (m, 2H), 2.00–2.03 (m, 2H), 2.10–2.12 (m, 2H), 2.37–2.44 (m, 8H), 2.51–2.59 (m, 5H), 2.62–2.72 (br, 3H), 2.76–2.80 (m, 1H), 3.00–3.28 (m, 12H), 3.36–3.41 (m, 2H), 3.42–3.50 (br, 2H), 3.53–3.57 (m, 1H), 3.60–3.64 (m, 1H), 3.68–3.70 (m, 1H), 3.74–3.81 (m, 2H), 3.83–3.96 (br, 1H), 4.35 (s, 2H), 5.09 (s, 2H), 5.54 (d, 1H, J = 15.4 Hz), 5.59 (d, 1H, J = 15.4 Hz), 6.85 (s, 1H), 7.10–7.13 (m, 2H), 7.17–7.20 (m, 3H), 7.27–7.30 (m, 2H), 7.32–7.34 (m, 4H), 7.51–7.56 (m, 2H), 7.60–7.62 (m, 2H), 7.73–7.77 (m, 3H), 8.32 (s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 21.5, 21.6, 29.4, 29.5, 30.1, 32.8, 34.6, 36.4, 37.4, 40.3, 40.6–40.7 (br), 41.4, 43.0, 43.8, 45.3, 47.8, 48.6, 49.1, 49.4, 49.6, 51.7, 61.8, 64.3, 66.3 (two signals overlapped), 66.7, 69.9, 70.4, 72.5, 119.0, 120.4, 121.5, 125.9, 126.0, 126.5, 127.1, 127.4, 127.7, 128.1, 129.0, 129.7, 129.8, 129.9 (two signals overlapped), 130.1, 130.4, 130.8, 131.5, 131.7, 133.2, 134.1, 134.2, 134.3, 135.1, 135.2, 136.6, 136.7, 137.7, 138.9, 141.4, 143.3, 143.8, 144.2, 144.7, 146.8, 169.4, 174.4; IR (KBr, cm^{-1}) 550, 1049, 1090, 1159, 1240, 1304, 1342, 1369, 1431, 1452, 1757, 1769, 2361, 2920; HRMS (ESI$^+$) *m*/*z* 1426.4427 ([M+Na]$^+$, $C_{74}H_{78}{}^{35}Cl_3N_{11}NaO_7S_2^+$ requires 1426.4485).

Platform–TESRA conjugate **S3a** and **S3b**

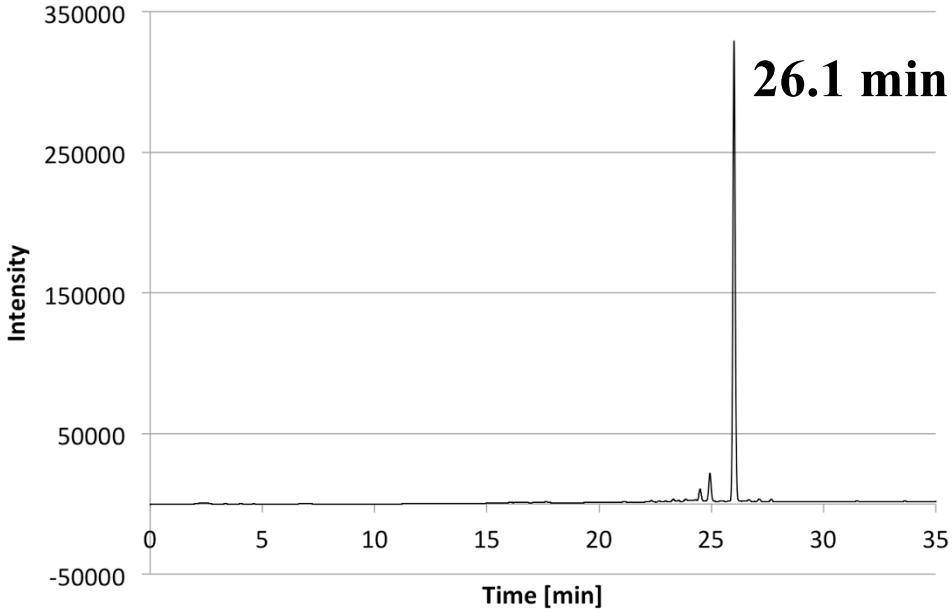


Since NMR analysis of these compounds gave complex spectra, the purity of each product was confirmed by analytical reverse phase HPLC [column: *SHISEIDO* CAPCELL PAK MG II (4.6 mm × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]

Platform–TESRA conjugate **S3a**

Purple solid; TLC R_f 0.45 (CH_2Cl_2/MeOH = 9/1); HPLC analysis: R*t* = 26.1 min; IR (KBr, cm^{-1}) 1028, 1171, 1180, 1246, 1275, 1337, 1416, 1452, 1466, 1591, 1649, 2090; HRMS (ESI^+) *m*/*z* 862.7138 ($[M+2Na]^{2+}$, $C_{79}H_{82}{}^{35}Cl_3N_{17}Na_2O_{13}S_3{}^{2+}$ requires 862.7145).

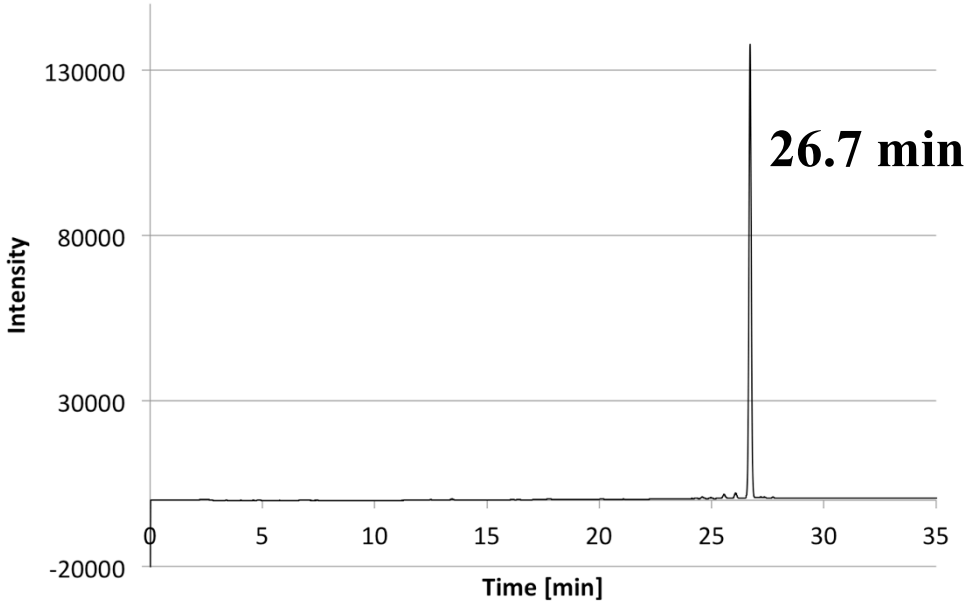
HPLC chart:



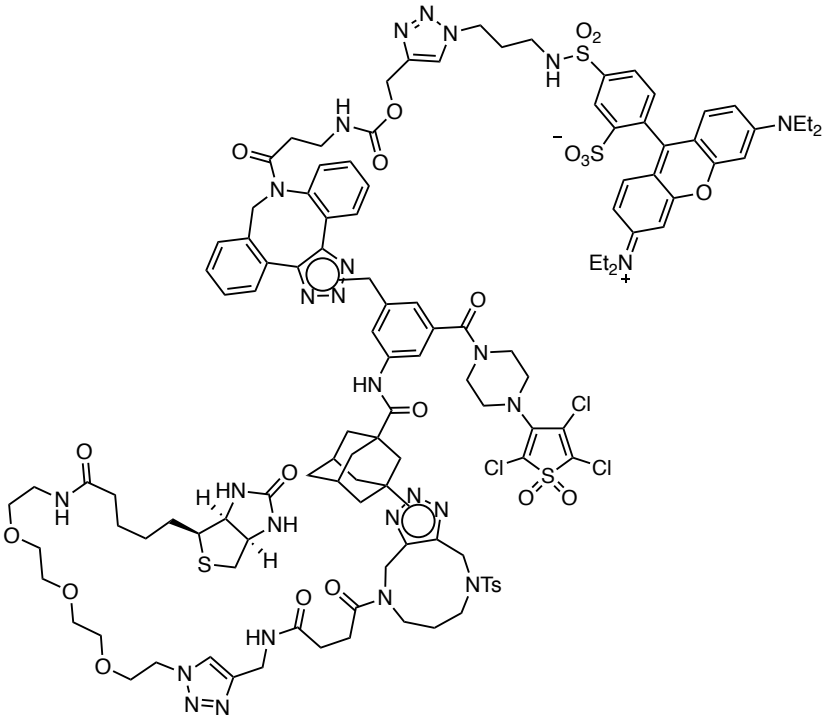
Platform–TESRA conjugate **S3b**

Purple solid; TLC R_f 0.45 (CH_2Cl_2/MeOH = 9/1); HPLC analysis: R*t* = 26.7 min; IR (KBr, cm^{-1}) 1028, 1180, 1246, 1275, 1337, 1416, 1450, 1466, 1591, 2100; HRMS (ESI^+) *m*/*z* 862.7146 ($[M+2Na]^{2+}$, $C_{79}H_{82}{}^{35}Cl_3N_{17}Na_2O_{13}S_3{}^{2+}$ requires 862.7145).

HPLC chart:



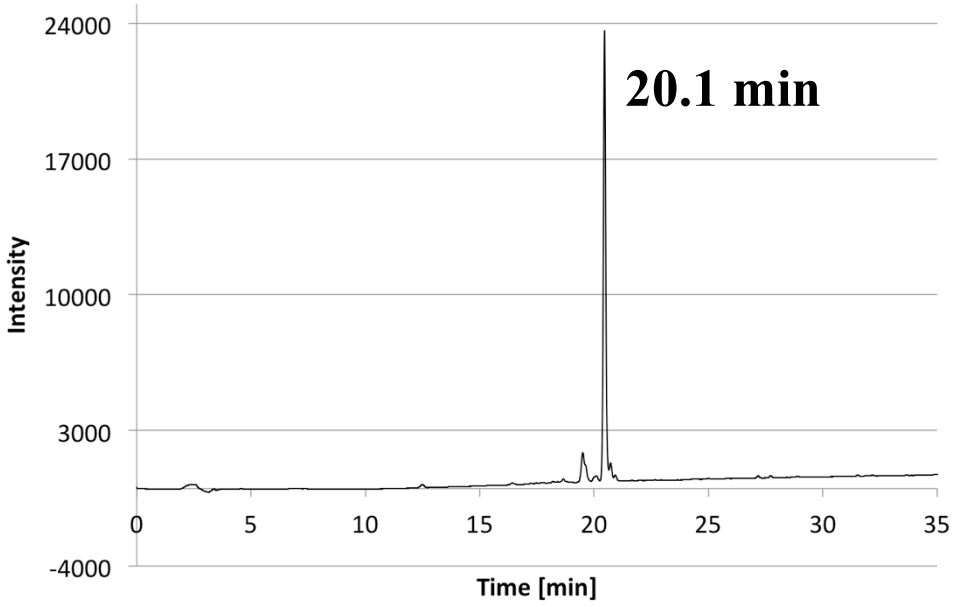
Platform–TESRA-biotin conjugate **S4aa**, **S4ab** and **S4ba**, **S4bb**



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 × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]

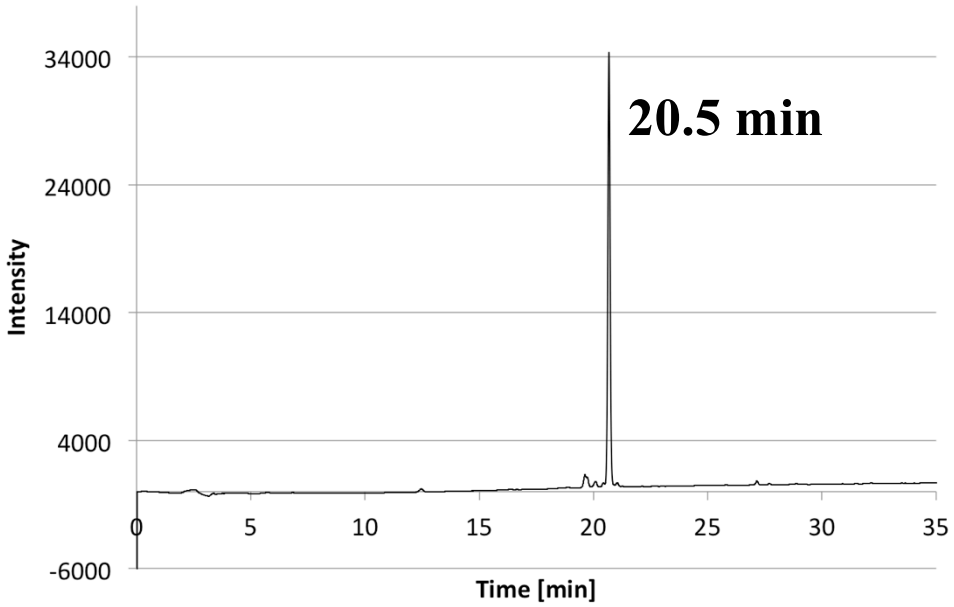
Platform–TESRA–biotin conjugate **S4aa**
Purple solid; TLC R_f 0.58 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: R*t* = 20.1 min; IR (KBr, cm^{-1}) 748, 1134, 1248, 1275, 1339, 1591, 2924; HRMS (ESI^+) *m/z* 1291.9006 ($[M+2Na]^{2+}$, $C_{118}H_{139}{}^{35}Cl_3N_{26}Na_2O_{22}S_5{}^{2+}$ requires 1291.9022).

HPLC chart:



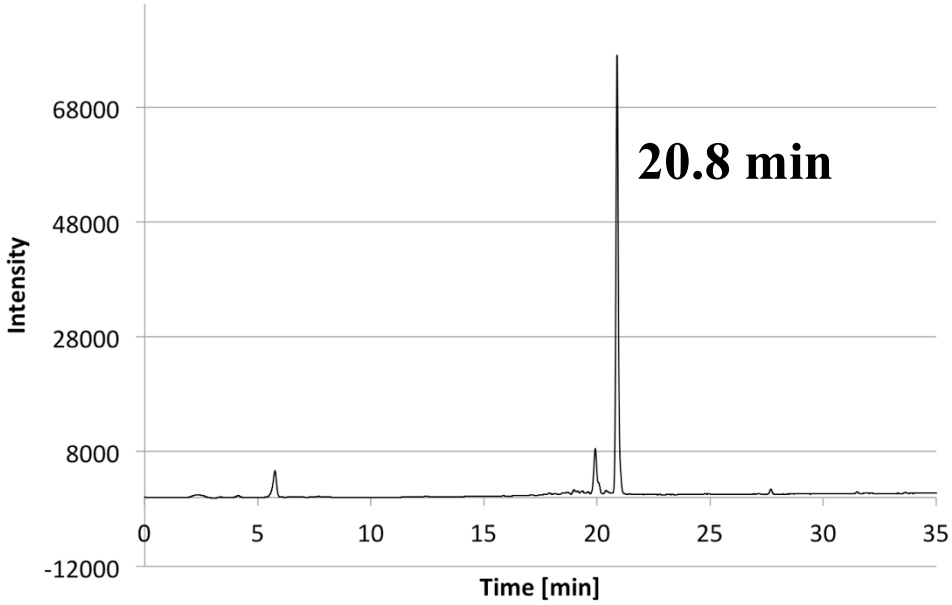
Platform–TESRA–biotin conjugate **S4ab**
Purple solid; TLC R_f 0.58 (CH_2Cl_2/MeOH = 5/1);HPLC analysis: R*t* = 20.5 min; IR (KBr, cm^{-1}) 750, 1076, 1134, 1159, 1180, 1259, 1339, 1416, 1454, 1591, 1649, 2924; HRMS (ESI^+) *m/z* 1291.9003 ($[M+2Na]^{2+}$, $C_{118}H_{139}{}^{35}Cl_3N_{26}Na_2O_{22}S_5{}^{2+}$ requires 1291.9022).

HPLC chart:



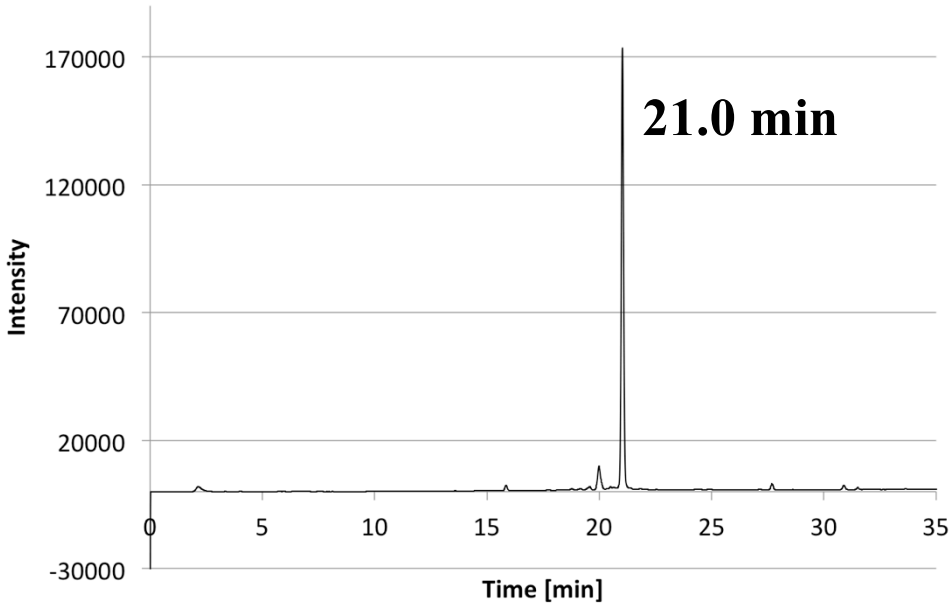
Platform–TESRA–biotin conjugate **S4ba**
Purple solid; TLC R_f 0.58 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: Rt = 20.8 min; IR (KBr, cm^{-1}) 748, 761, 1134, 1248, 1275, 1339, 1591, 1649, 1721, 2926; HRMS (ESI^+) m/z 1291.9009 ($[M+2Na]^{2+}$, $C_{118}H_{139}{}^{35}Cl_3N_{26}Na_2O_{22}S_5{}^{2+}$ requires 1291.9022).

HPLC chart:

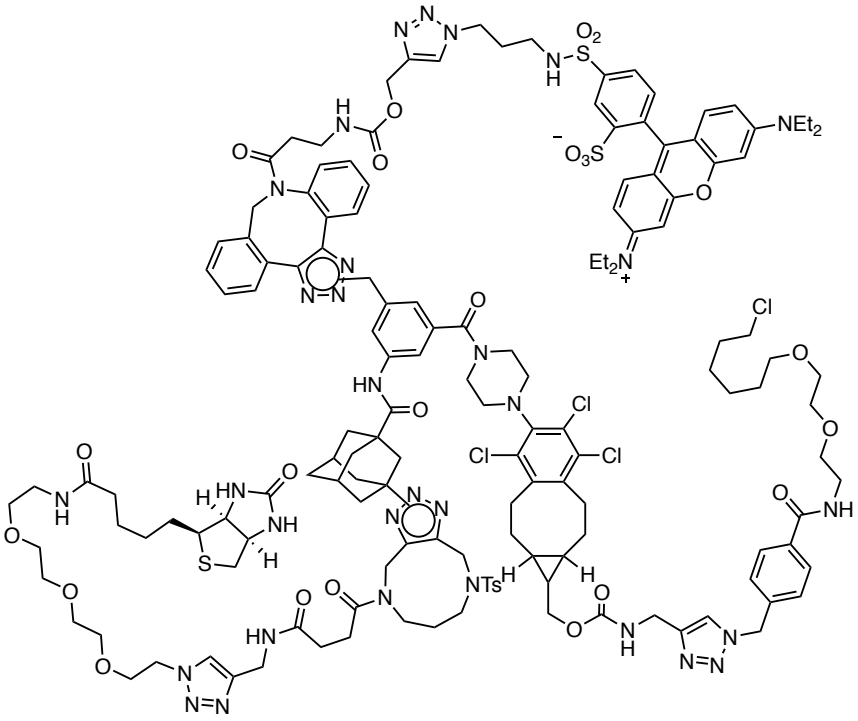


Platform–TESRA–biotin conjugate **S4bb**
Purple solid; TLC R_f 0.58 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: Rt = 21.0 min; IR (KBr, cm^{-1}) 750, 762, 1180, 1248, 1260, 1275, 1339, 1591, 1722, 2853, 2924; HRMS (ESI^+) m/z 1291.8985 ($[M+2Na]^{2+}$, $C_{118}H_{139}{}^{35}Cl_3N_{26}Na_2O_{22}S_5{}^{2+}$ requires 1291.9022).

HPLC chart:



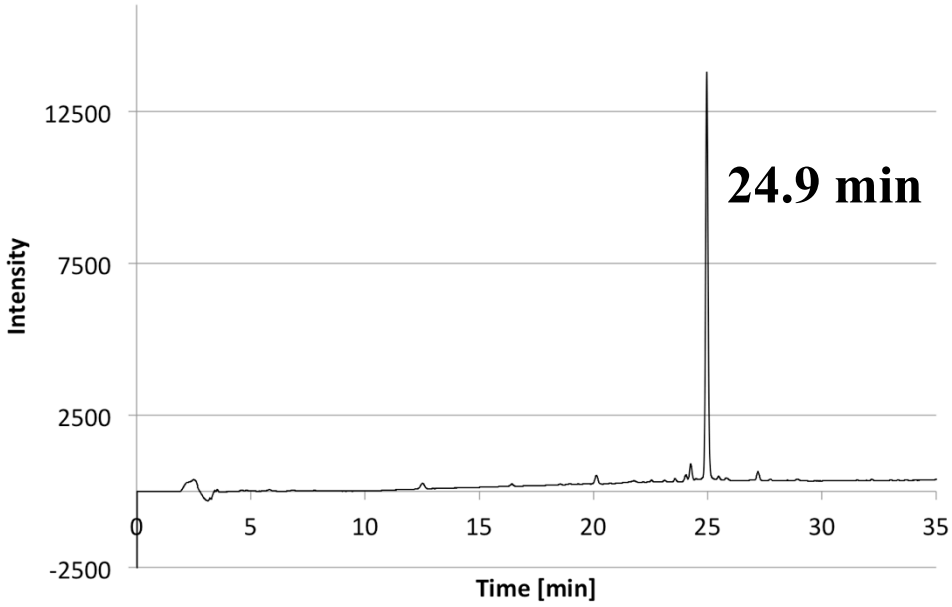
Platform–TESRA–biotin–HaloTag-ligand conjugates **29aa**, **29ab**, **29ba**, and **29bb**



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 × 150 mm); mobile phase: CH_3CN:H_2O = 40:60 (0–5 min), linear gradient from 40:60 to 99:1 (5–30 min); flow rate: 1.00 mL/min; detection: UV at 254 nm]

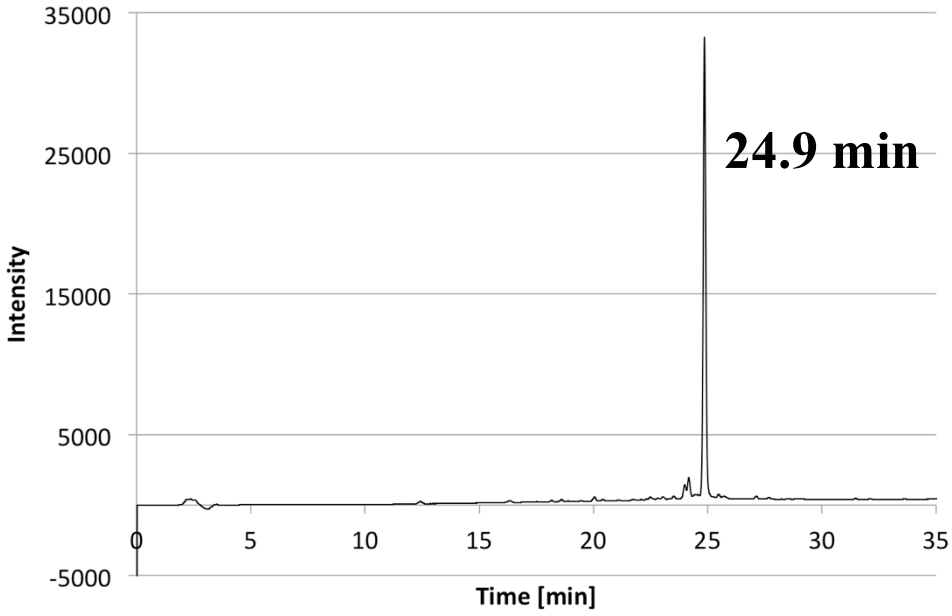
Platform–TESRA–biotin–HaloTag-ligand conjugate **29aa**
Purple solid; TLC R_f 0.58 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: R*t* = 24.9 min; IR (KBr, cm^{-1}) 1180, 1246, 1275, 1339, 1416, 1454, 1591, 1643, 1714, 2855; HRMS (ESI^+) *m*/*z* 1566.0672 ($[M+2Na]^{2+}$, $C_{150}H_{183}{}^{35}Cl_4N_{31}Na_2O_{25}S_4{}^{2+}$ requires 1566.0711).

HPLC chart:



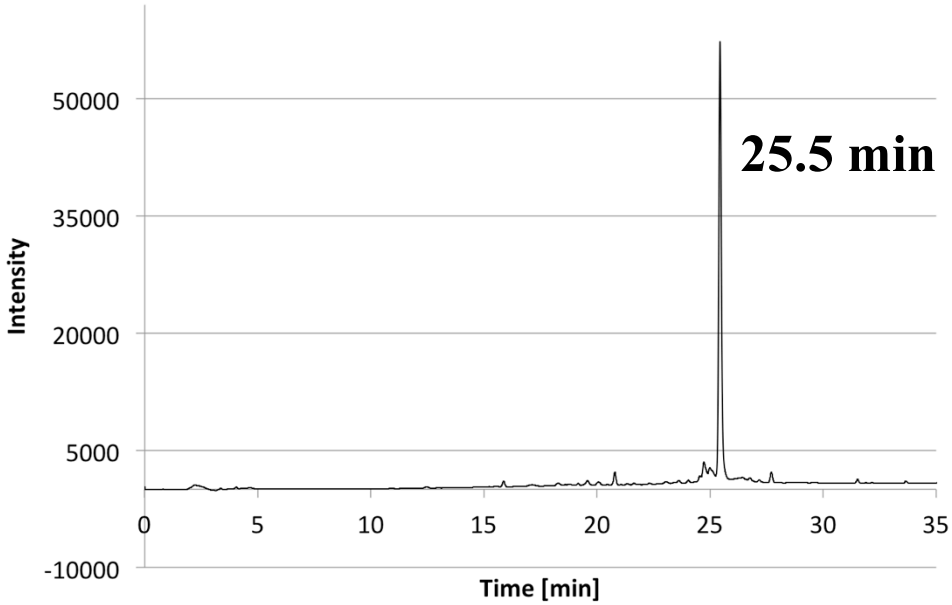
Platform–TESRA–biotin–HaloTag ligand conjugate **29ab**
Purple solid; TLC R_f 0.58 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: Rt = 24.9 min; IR (KBr, cm^{-1}) 1076, 1136, 1159, 1180, 1246, 1275, 1339, 1420, 1591, 1719, 2856; HRMS (ESI^+) m/z 1566.0676 ($[M+2Na]^{2+}$, $C_{150}H_{183}{}^{35}Cl_4N_{31}Na_2O_{25}S_4{}^{2+}$ requires 1566.0711).

HPLC chart:



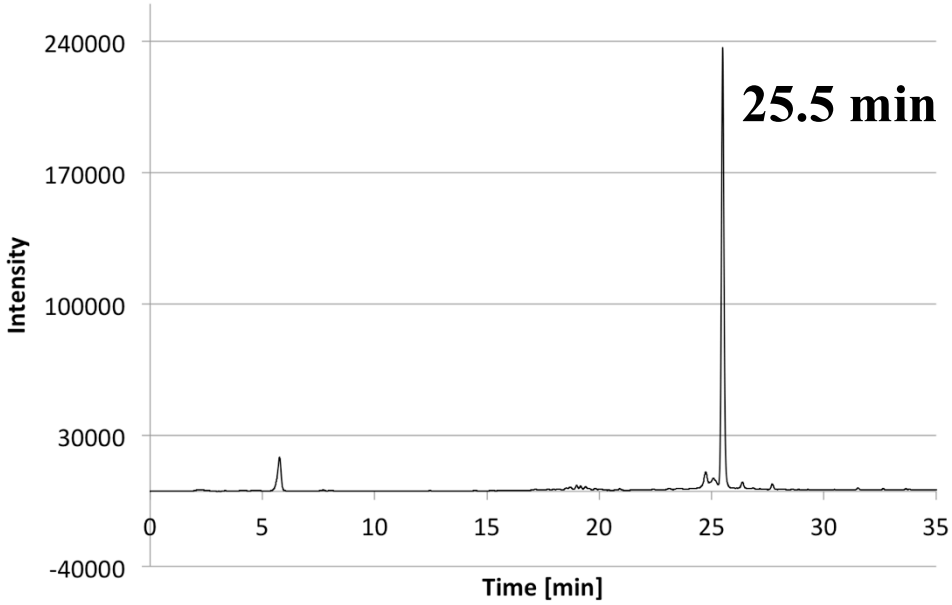
Platform–TESRA–biotin–HaloTag-ligand conjugate **29ba**
Purple solid; TLC R_f 0.58 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: Rt = 25.5 min; IR (KBr, cm^{-1}) 748, 1082, 1136, 1161, 1182, 1261, 1275, 1339, 1420, 1591, 1717, 1734, 2855; HRMS (ESI^+) m/z 1566.0689 ($[M+2Na]^{2+}$, $C_{150}H_{183}{}^{35}Cl_4N_{31}Na_2O_{25}S_4{}^{2+}$ requires 1566.0711).

HPLC chart:



Platform–TESRA–biotin–HaloTag-ligand conjugate **29bb**
Purple solid; TLC R_f 0.58 (CH_2Cl_2/MeOH = 5/1); HPLC analysis: Rt = 25.5 min; IR (KBr, cm^{-1}) 748, 1080, 1132, 1267, 1275, 1339, 1591, 1732, 2340, 2360, 2855; HRMS (ESI^+) m/z 1566.0716 ($[M+2Na]^{2+}$, $C_{150}H_{183}{}^{35}Cl_4N_{31}Na_2O_{25}S_4{}^{2+}$ requires 1566.0711).

HPLC chart:



References for Supporting Information

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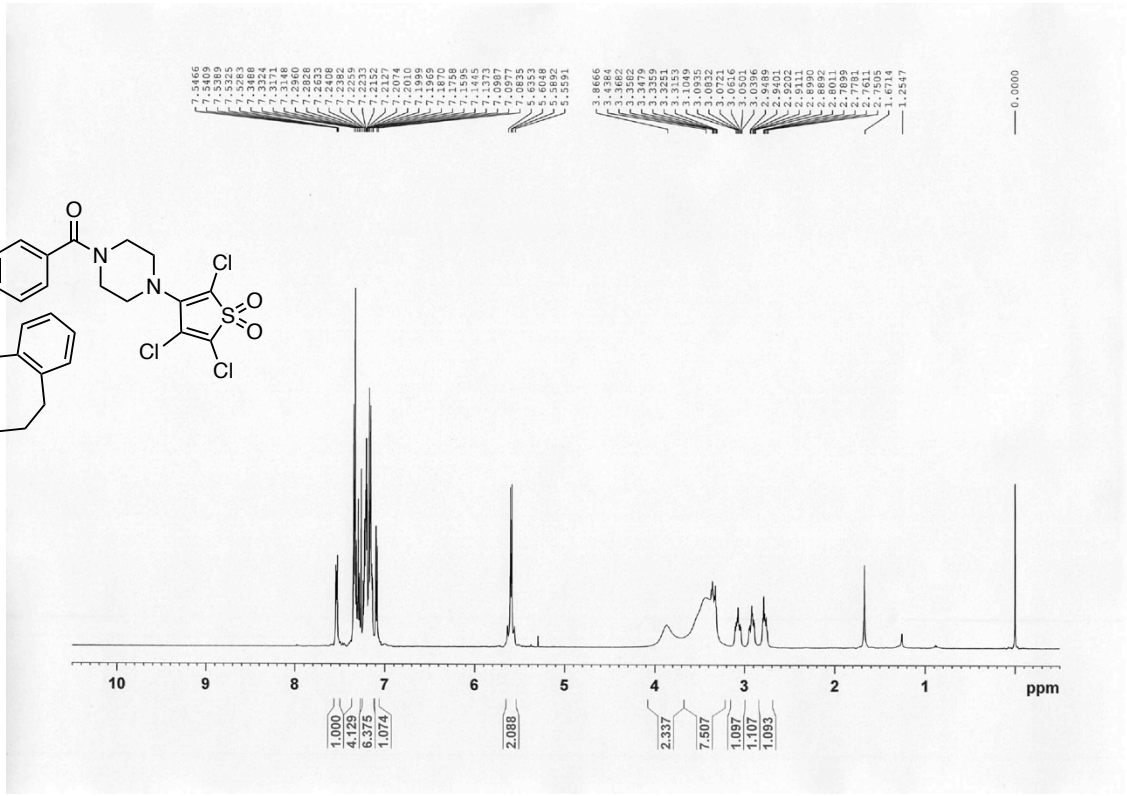
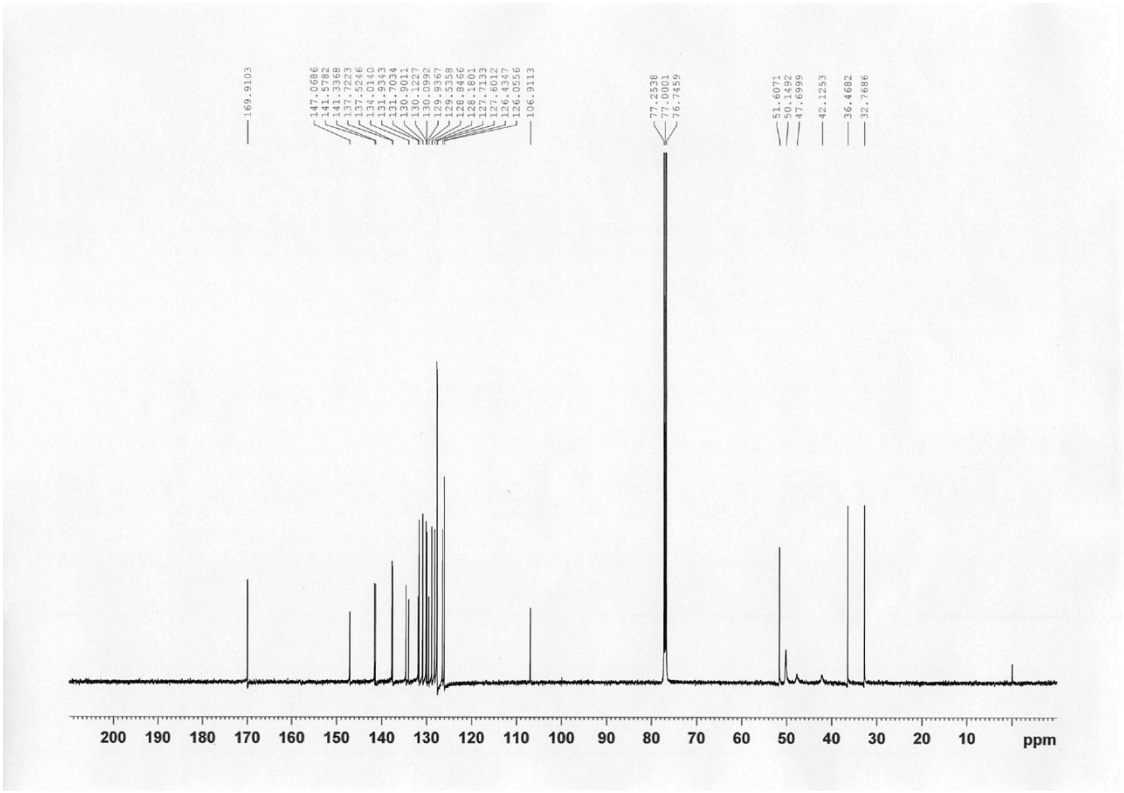
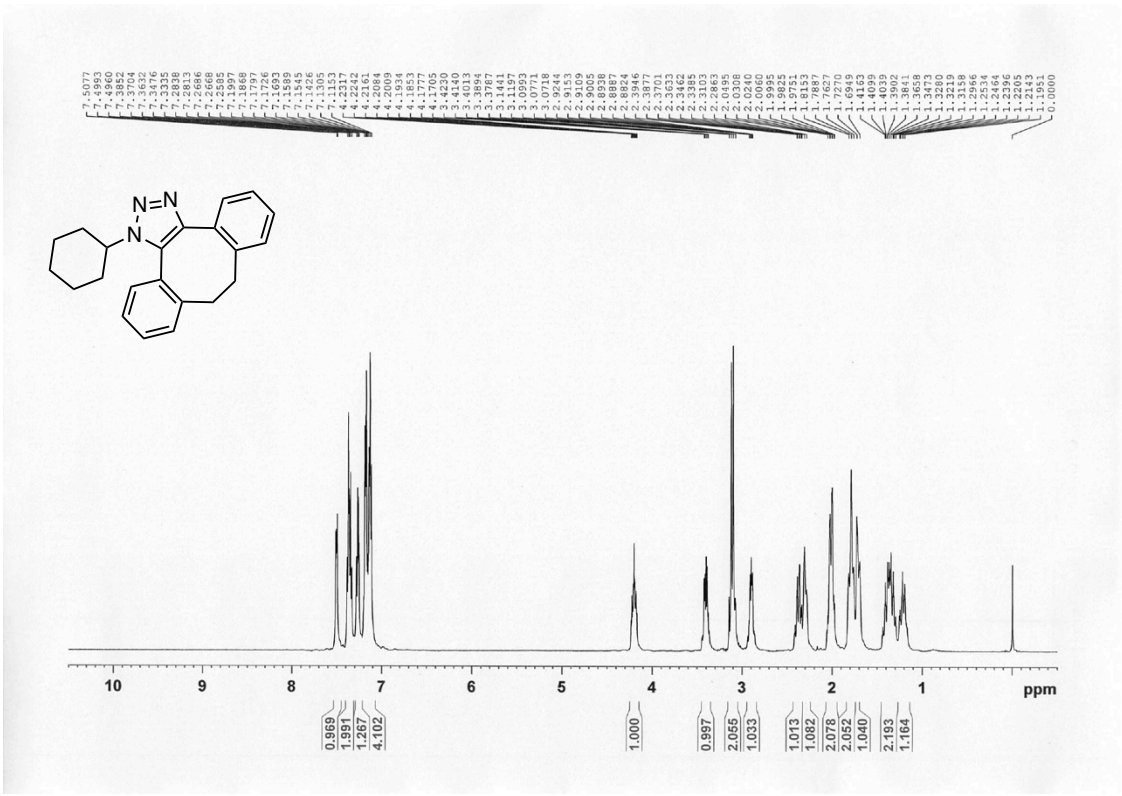
^{1}H and ^{13}C NMR Spectra of Compounds

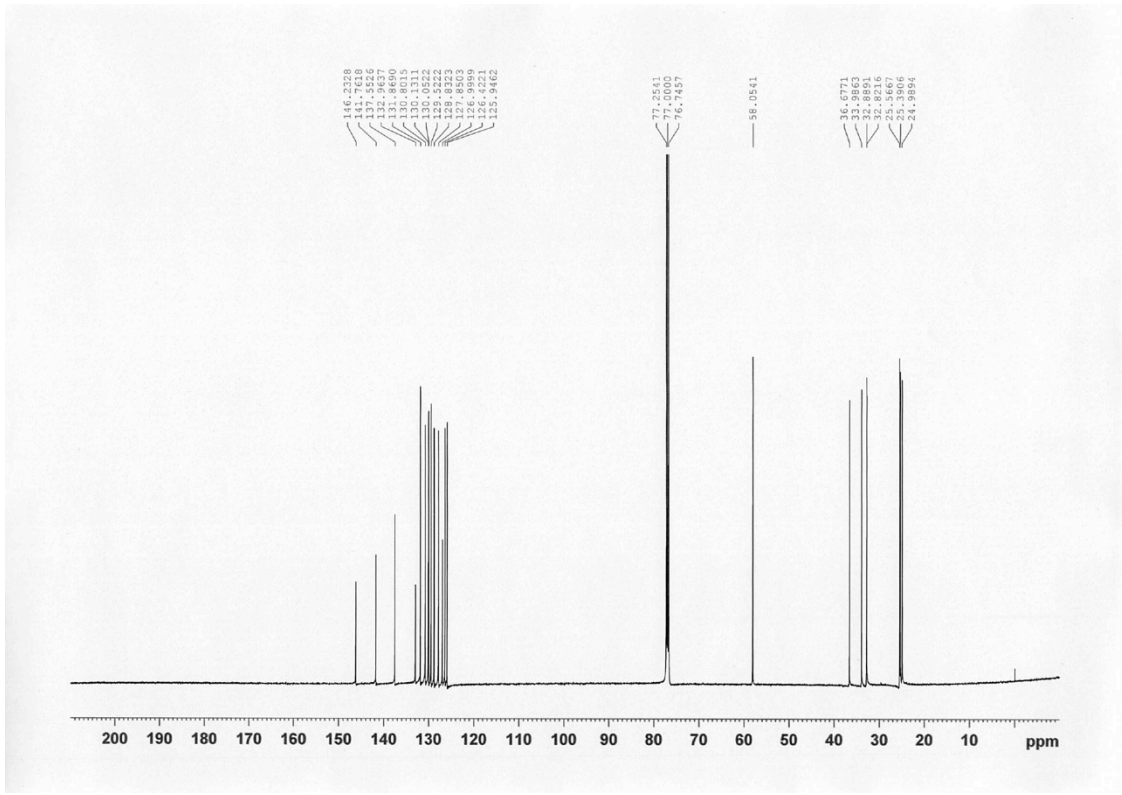
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **9** ($CDCl_3$)



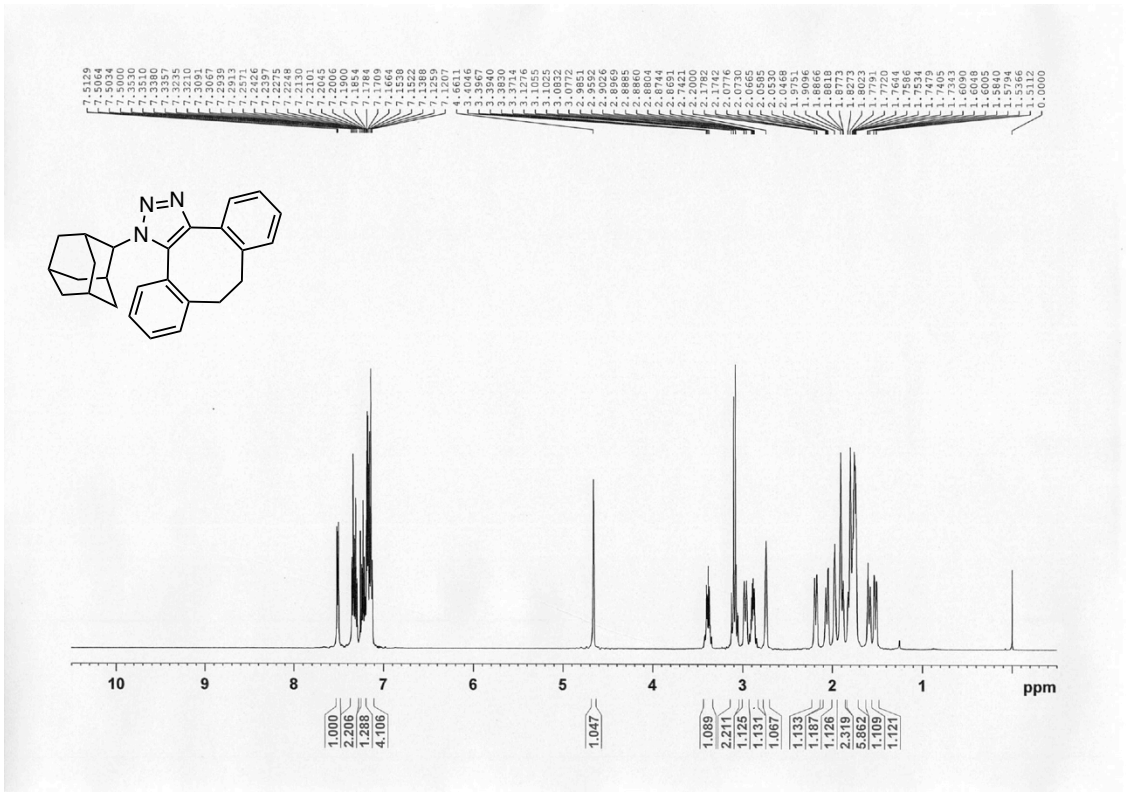


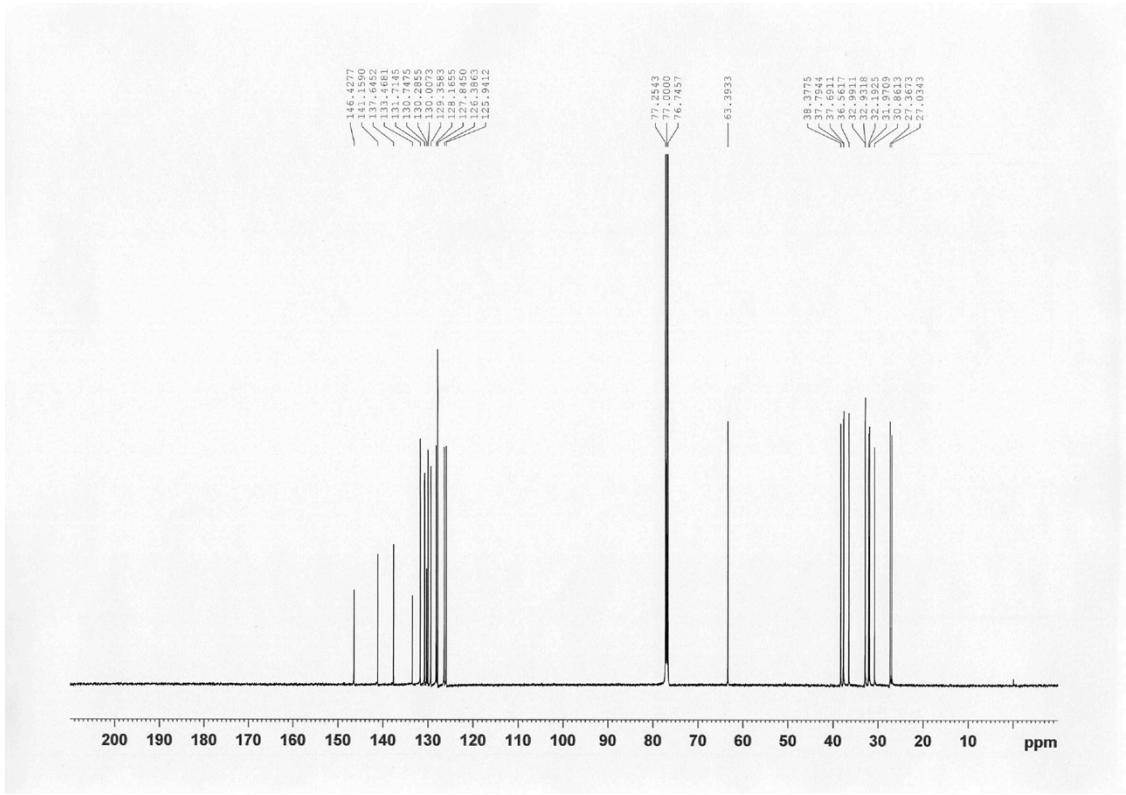
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **11b** ($CDCl_3$)



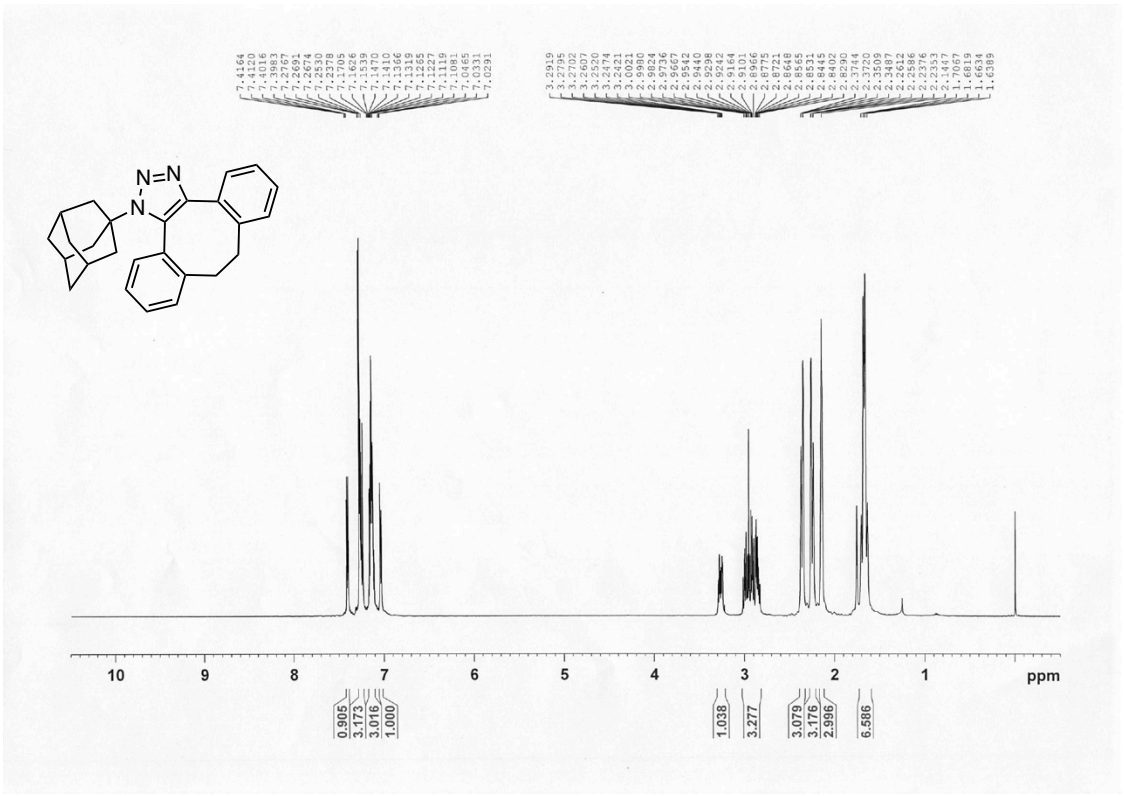


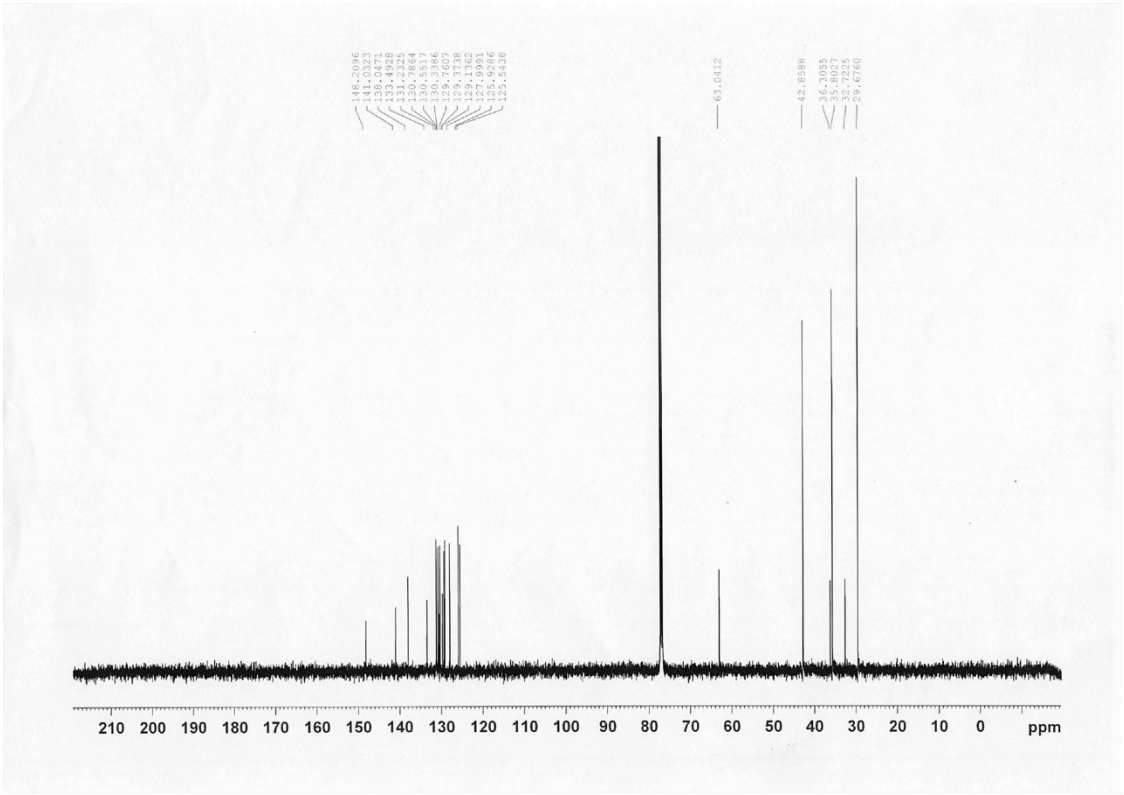
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **11c** ($CDCl_3$)



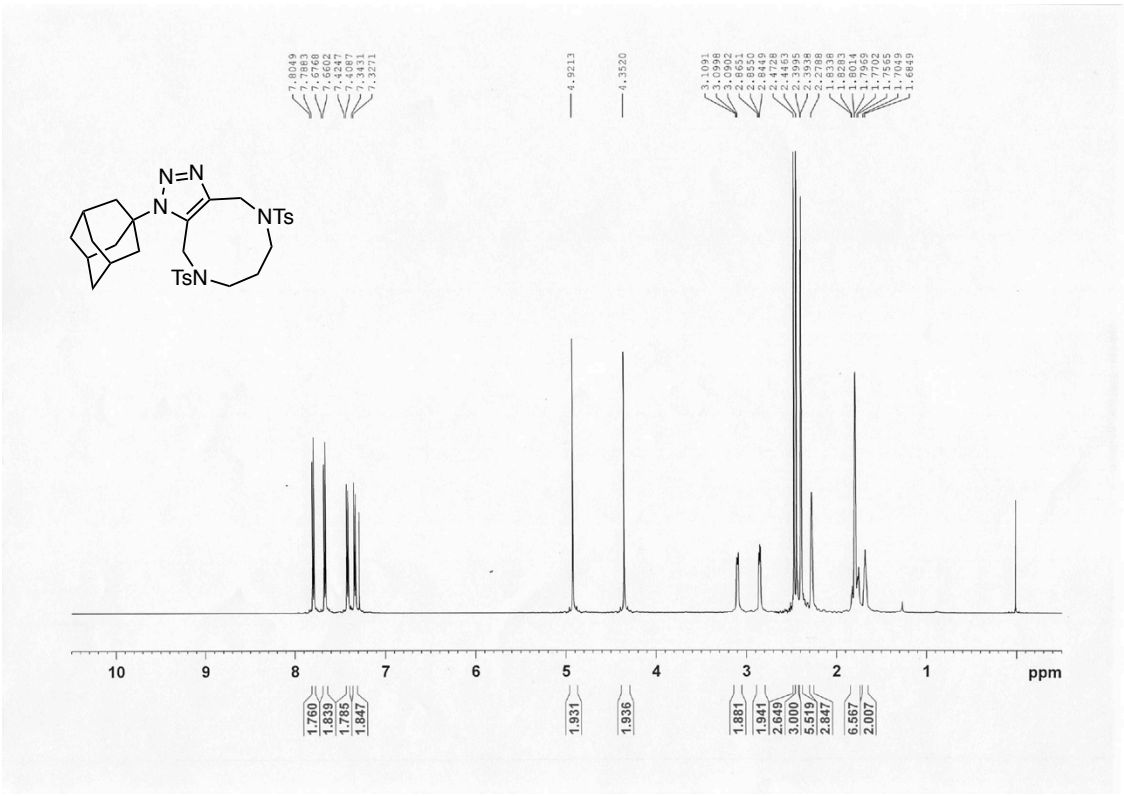


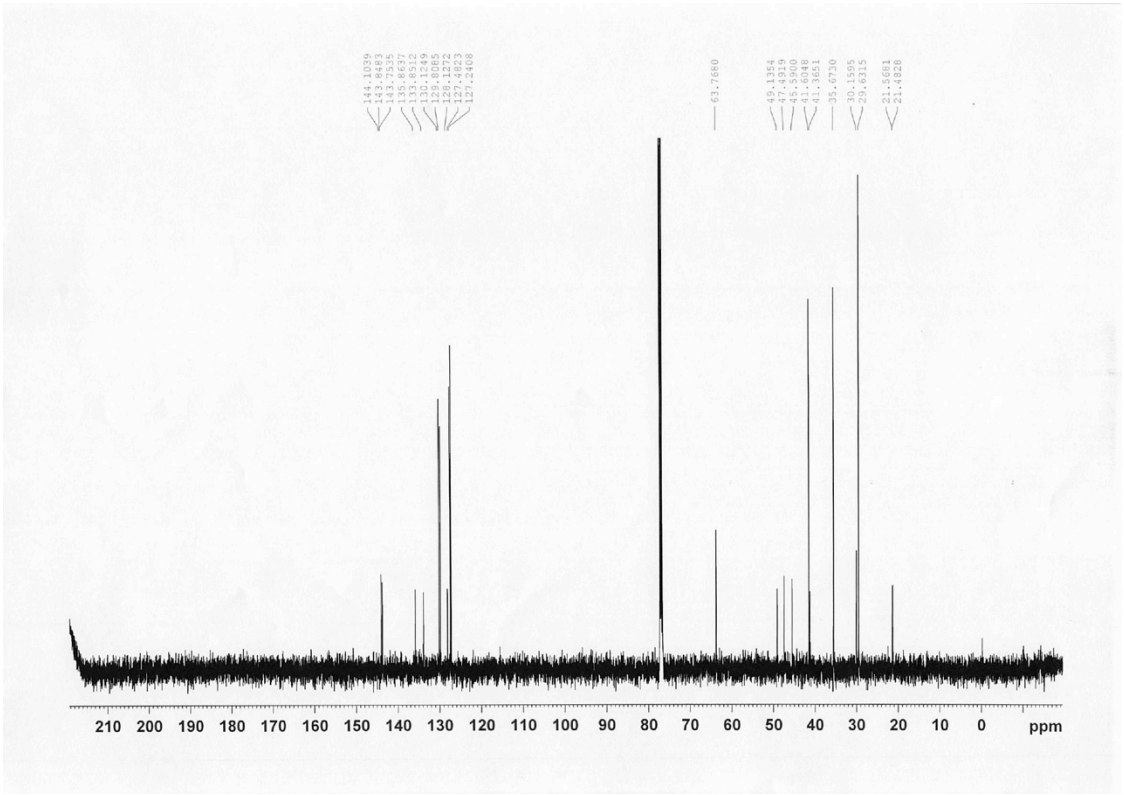
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **11d** ($CDCl_3$)



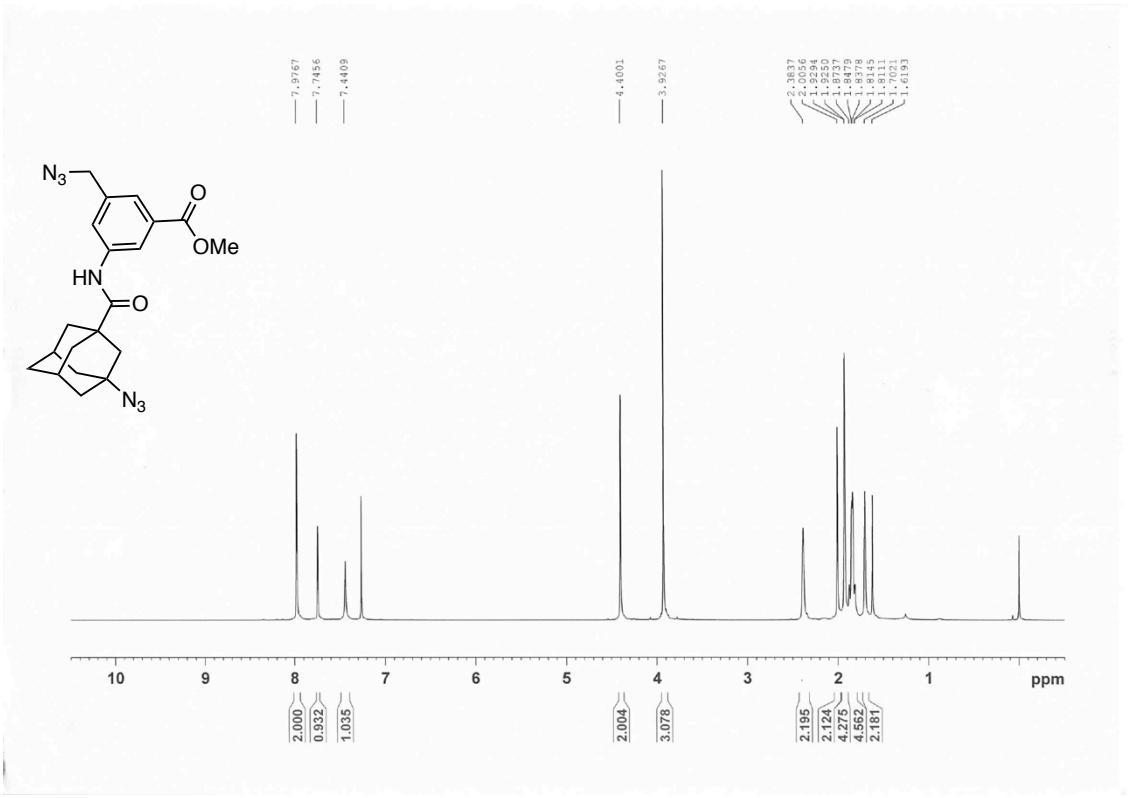


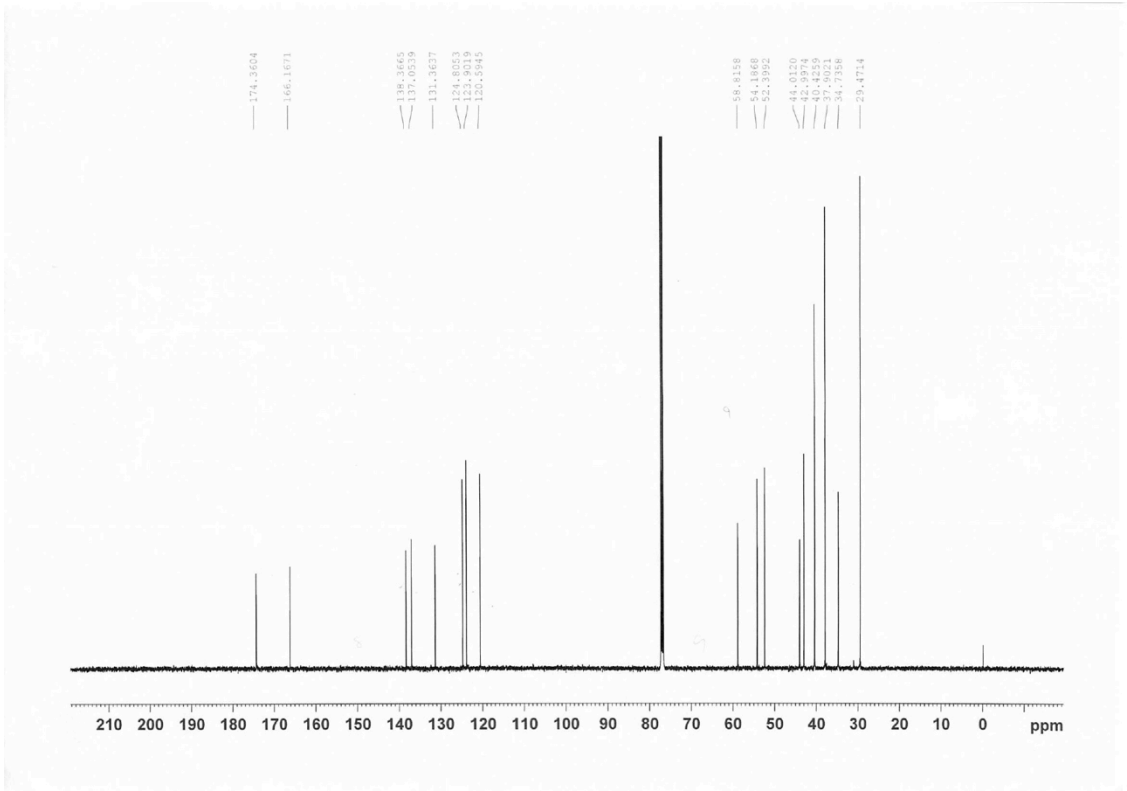
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **15** ($CDCl_3$)



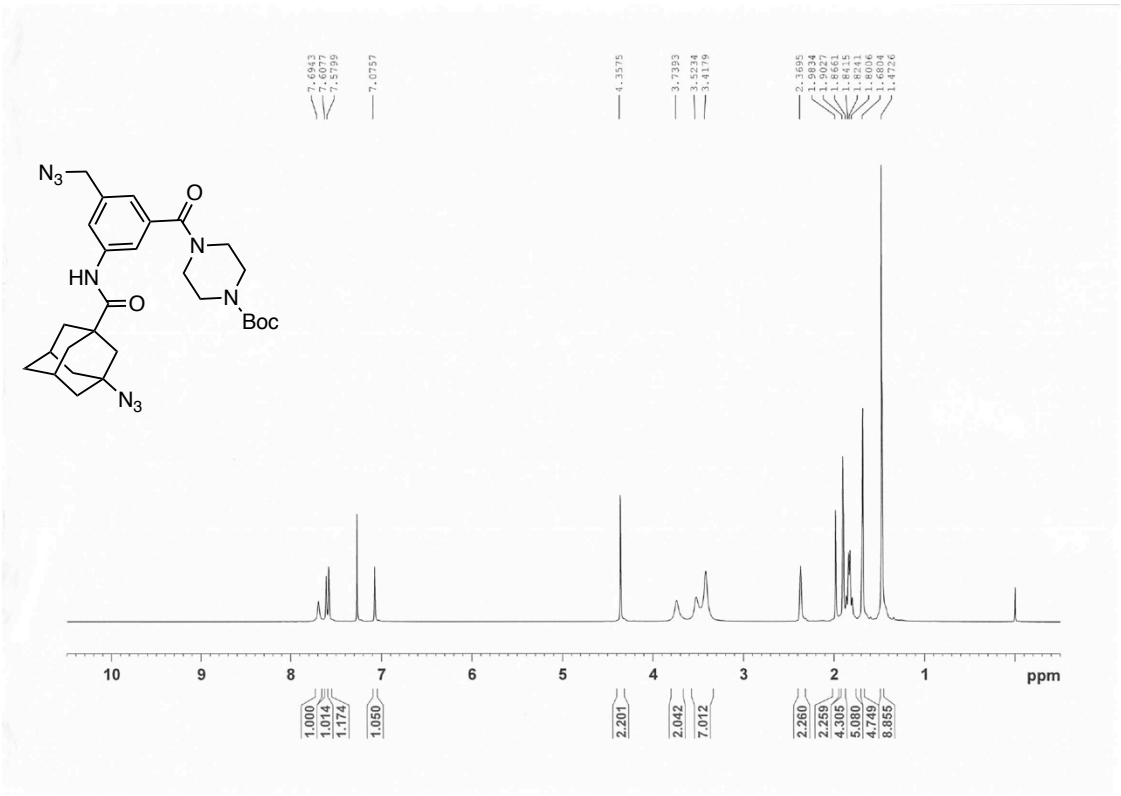


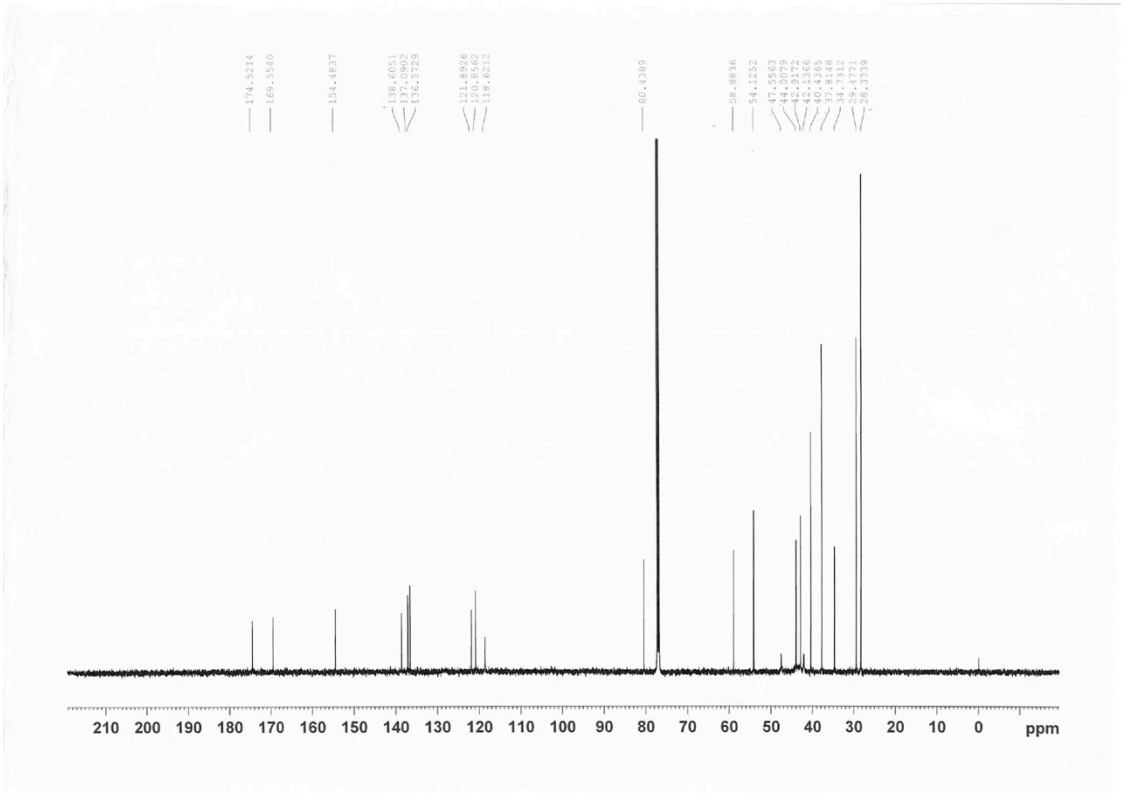
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **20** ($CDCl_3$)



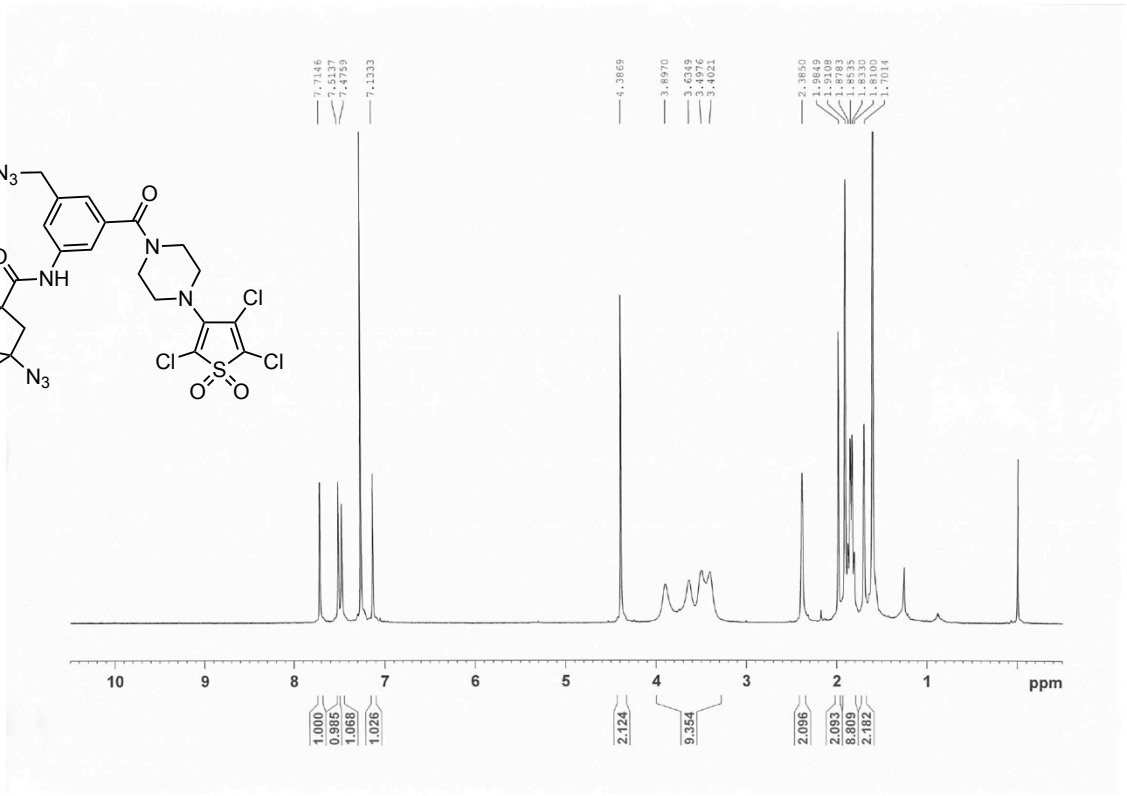


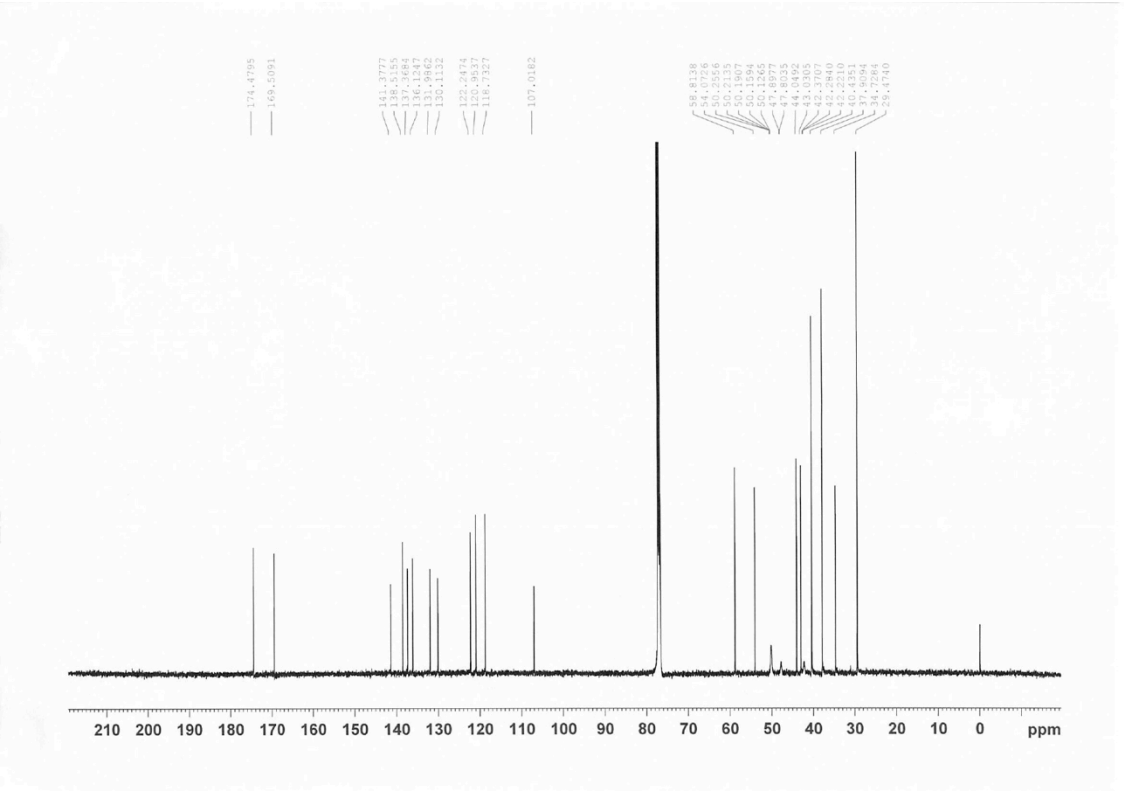
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **22** ($CDCl_3$)



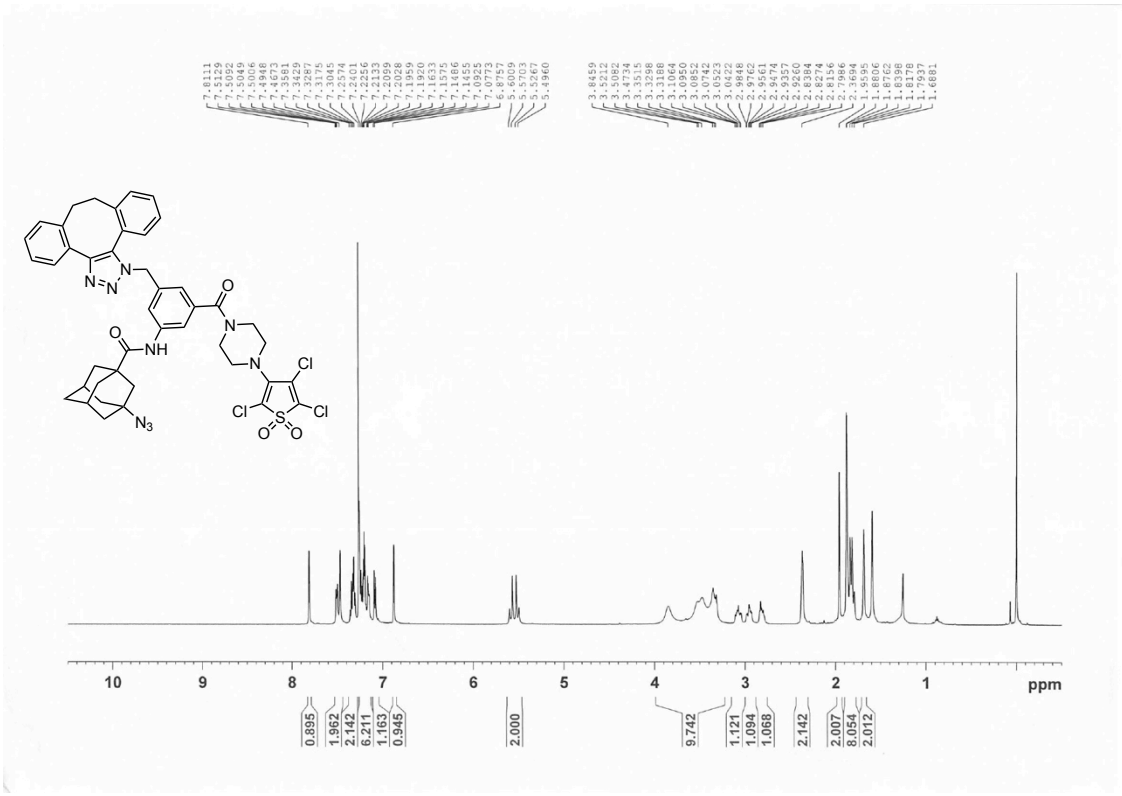


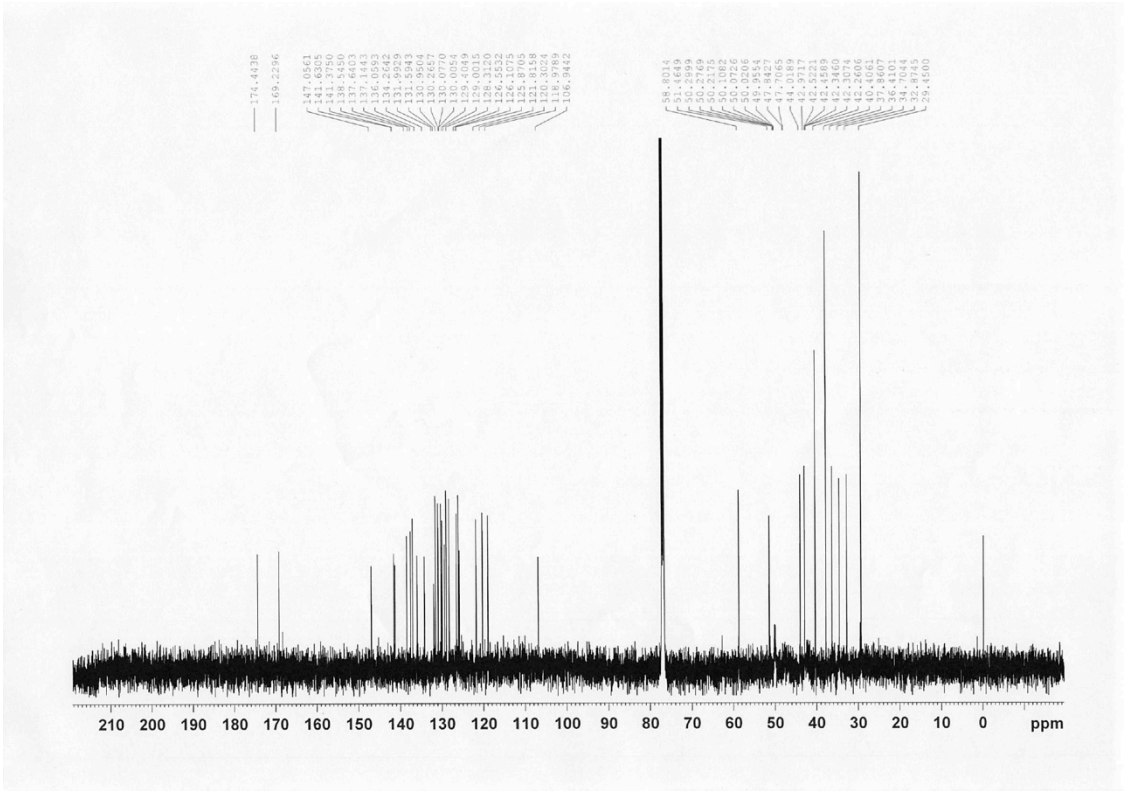
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **24** ($CDCl_3$)



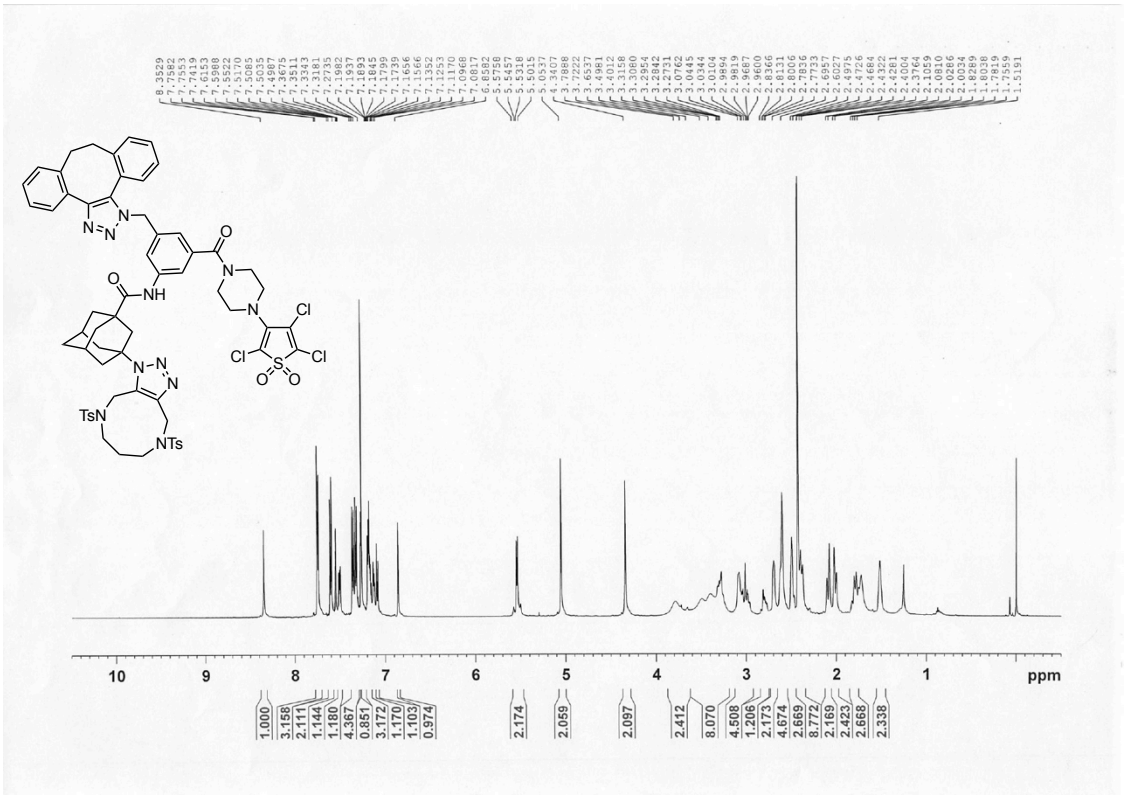


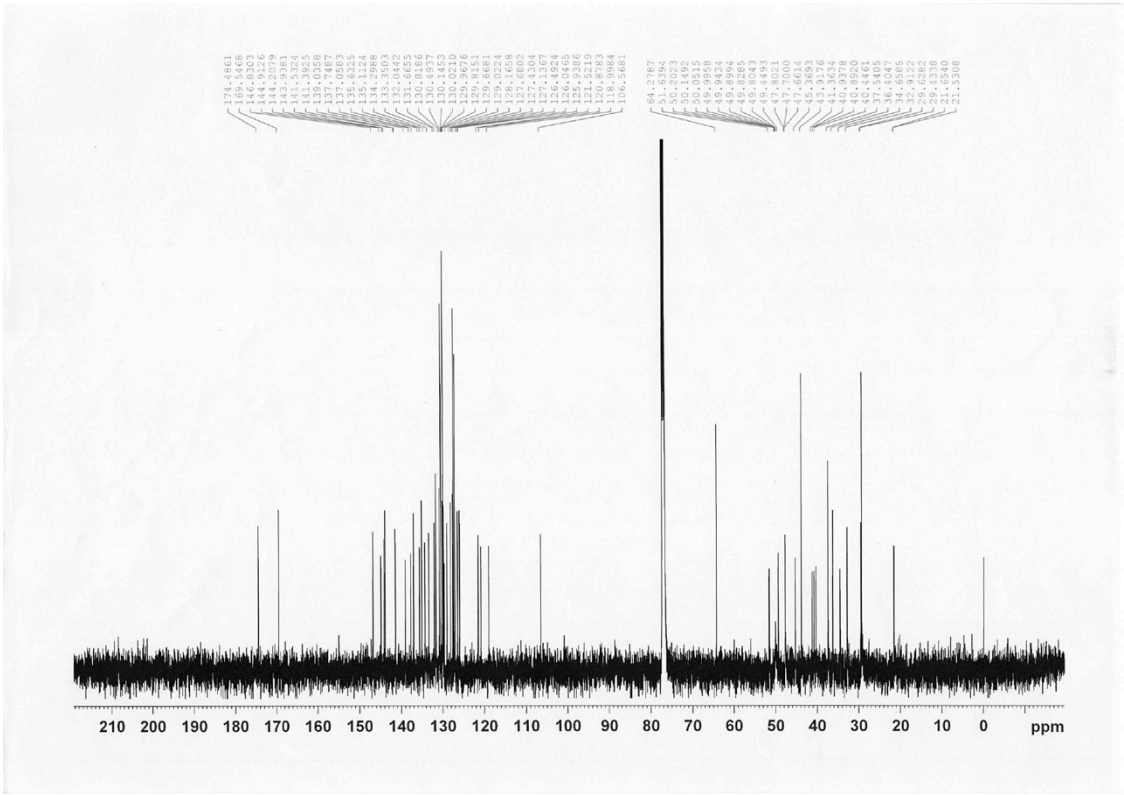
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **S1** ($CDCl_3$)



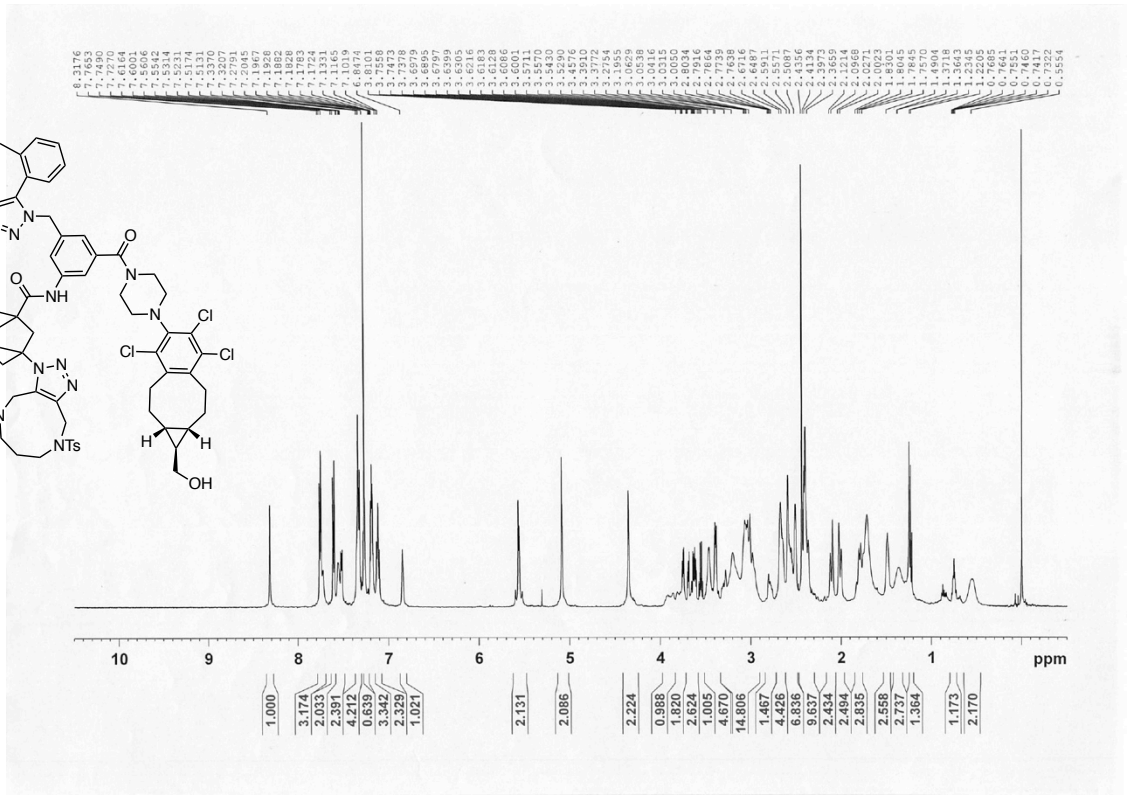


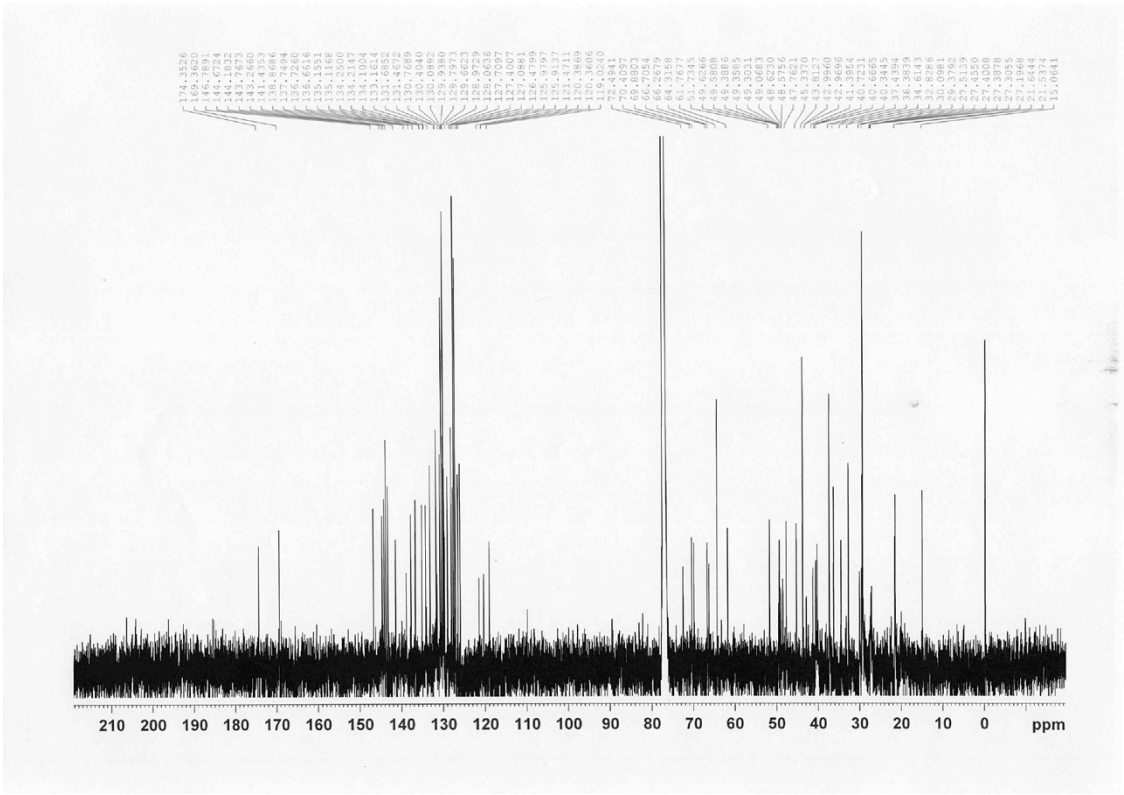
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **S2** ($CDCl_3$)



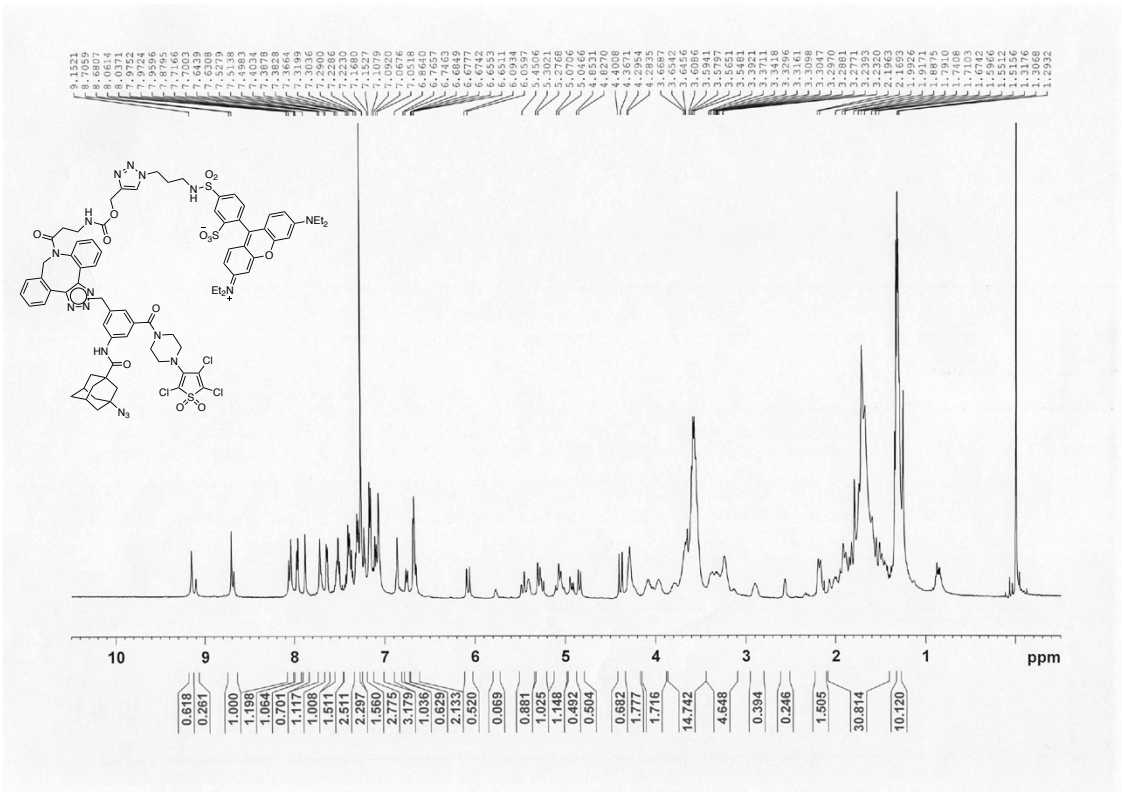


^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra of **26** ($CDCl_3$)

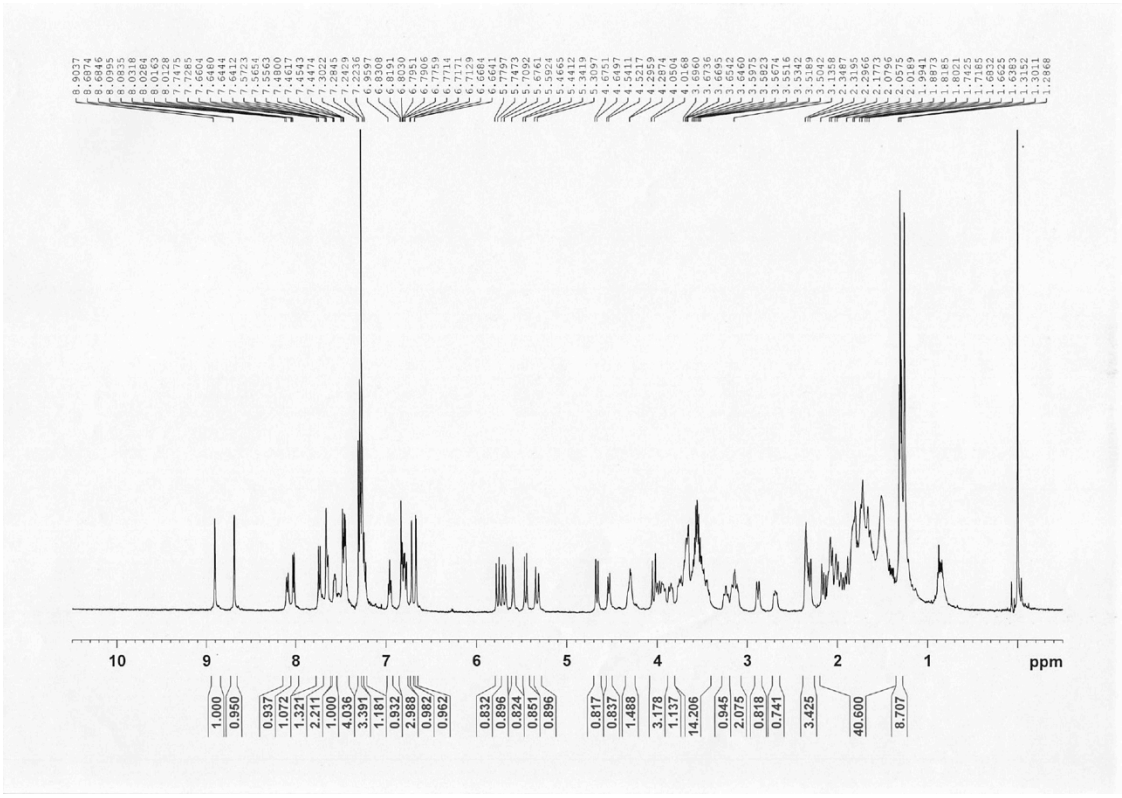




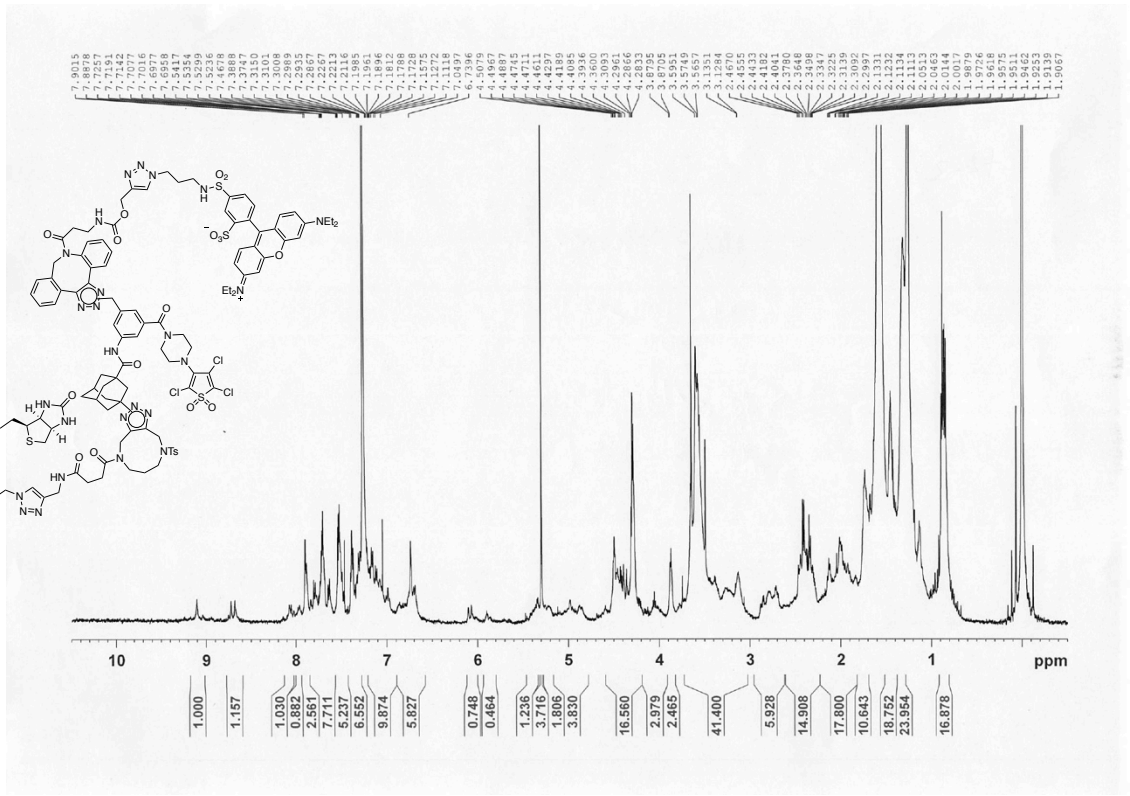
^{1}H NMR (500 MHz) spectrum of **S3a** ($CDCl_3$)



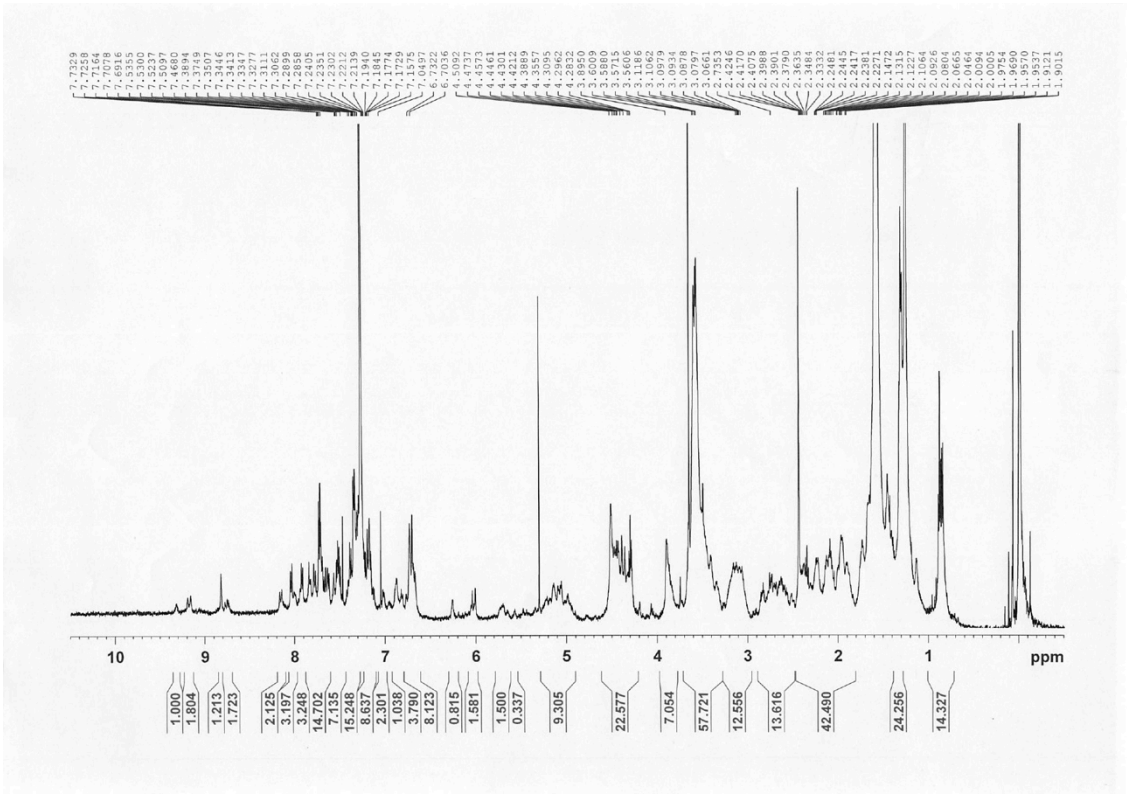
^{1}H NMR (500 MHz) spectrum of **S3b** ($CDCl_3$)



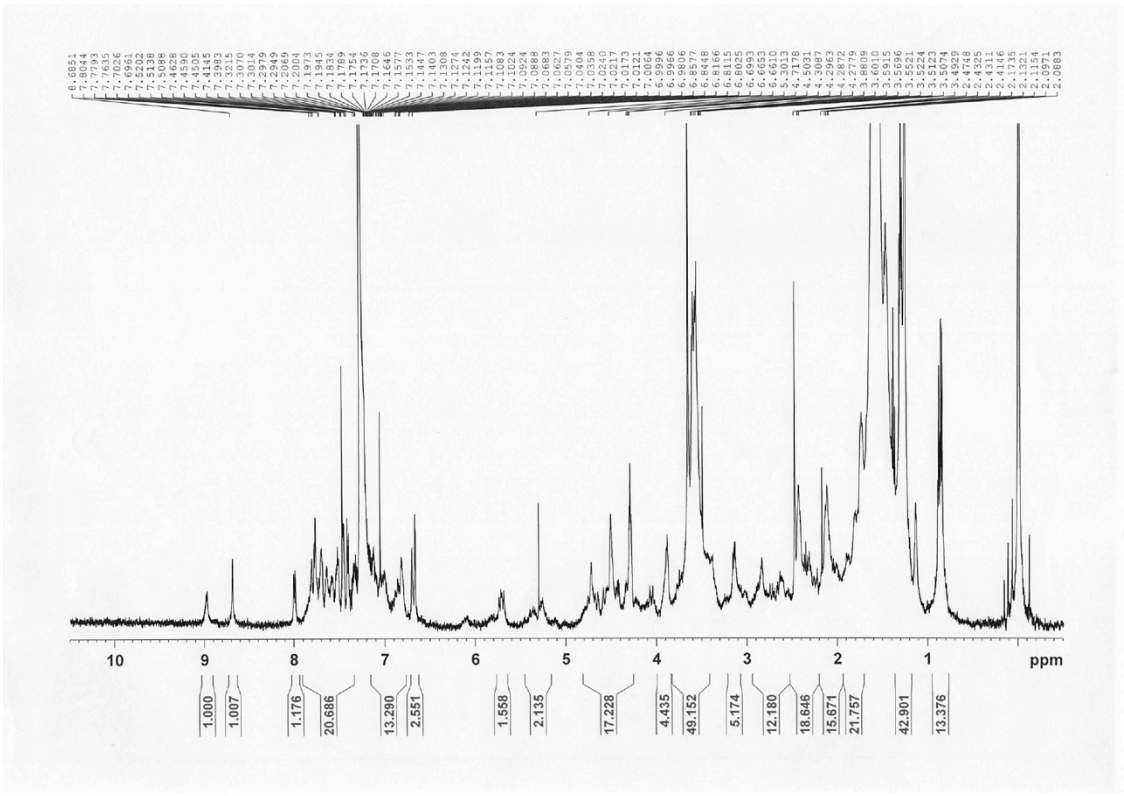
^{1}H NMR (500 MHz) spectrum of **S4aa** ($CDCl_3$)



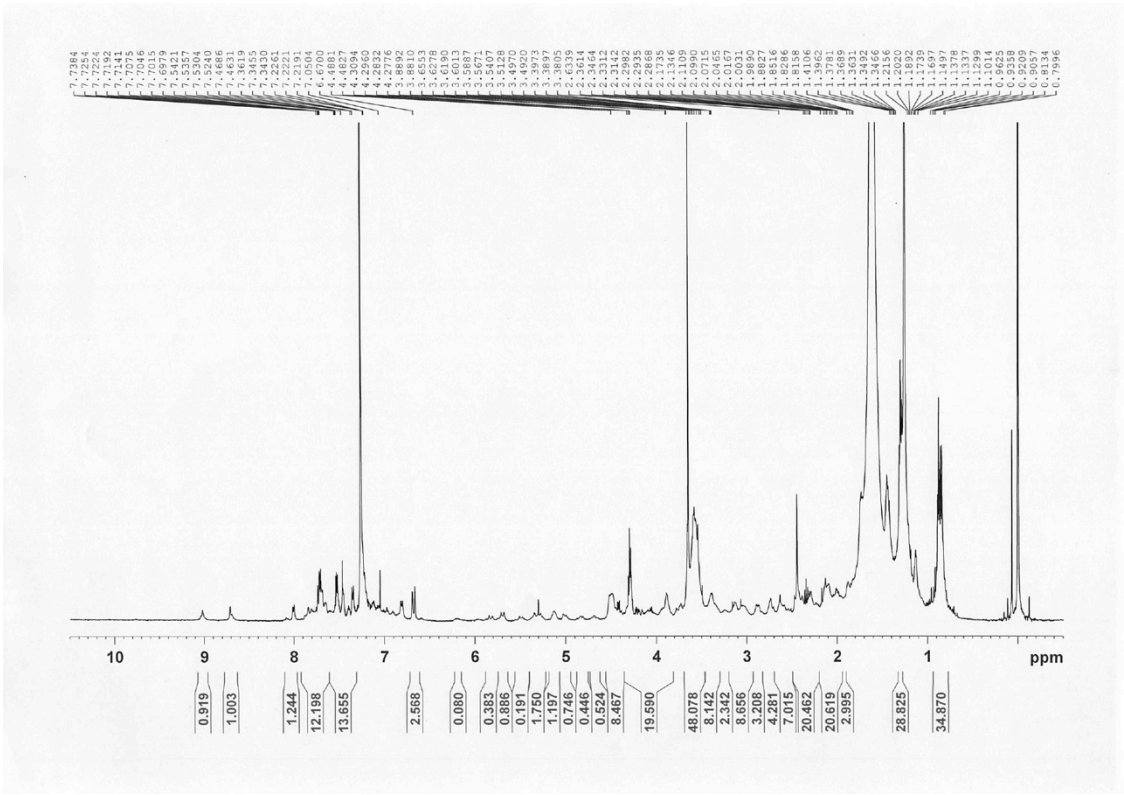
^{1}H NMR (500 MHz) spectrum of **S4ab** ($CDCl_3$)



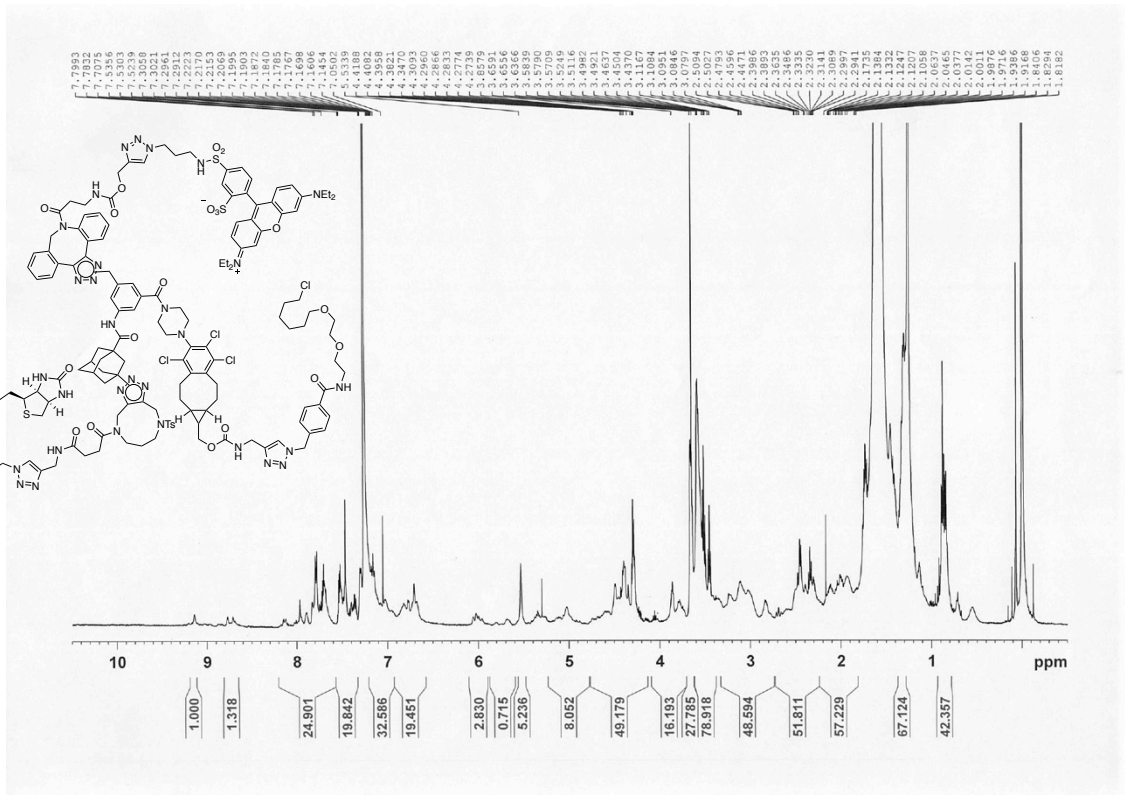
^{1}H NMR (500 MHz) spectrum of **S4ba** ($CDCl_3$)



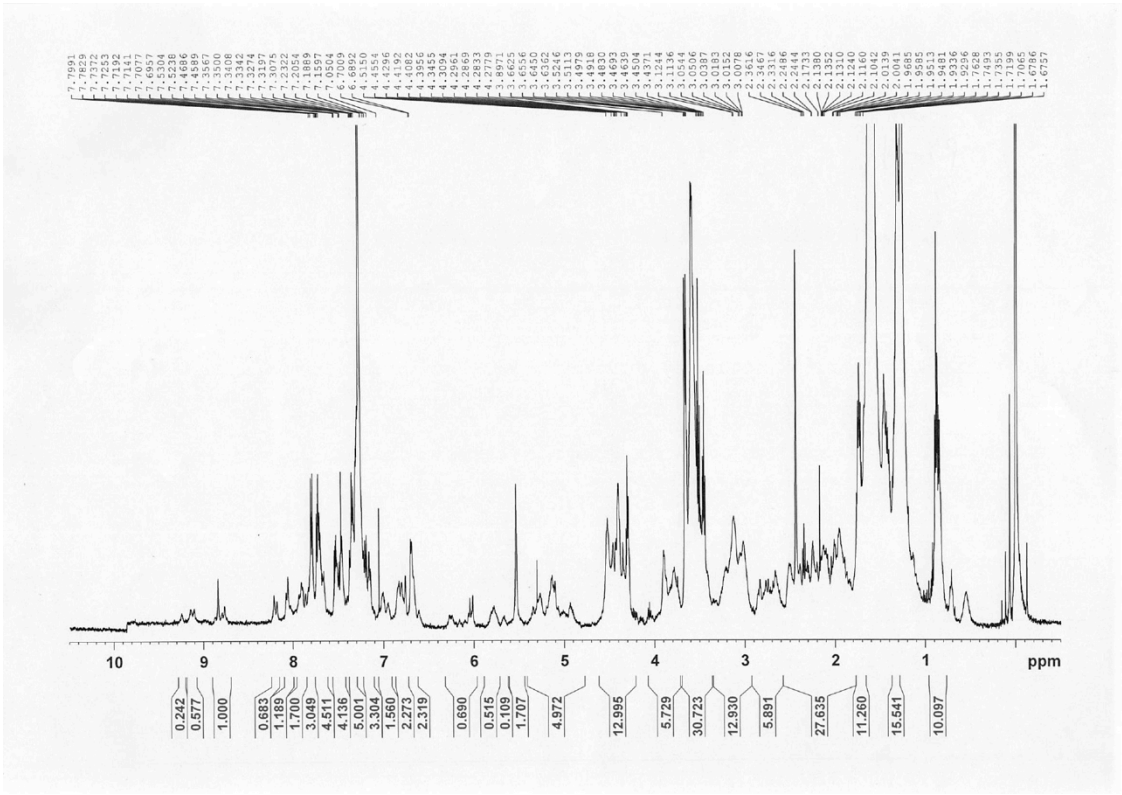
^{1}H NMR (500 MHz) spectrum of **S4bb** ($CDCl_3$)



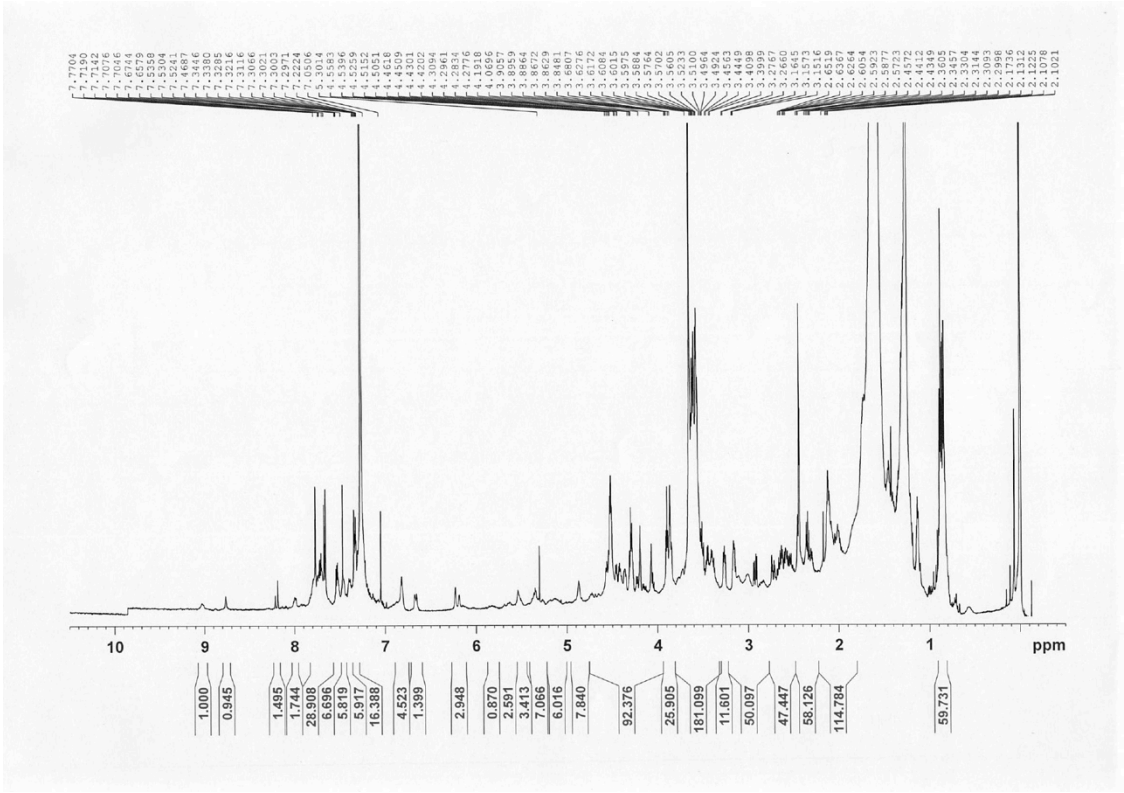
^{1}H NMR (500 MHz) spectrum of **29aa** ($CDCl_3$)



^{1}H NMR (500 MHz) spectrum of **29ab** ($CDCl_3$)



^{1}H NMR (500 MHz) spectrum of **29ba** ($CDCl_3$)



^{1}H NMR (500 MHz) spectrum of **29bb** ($CDCl_3$)