~ Supporting Information ~

Reduction Triggered *In Situ* Polymerization in Living Mice

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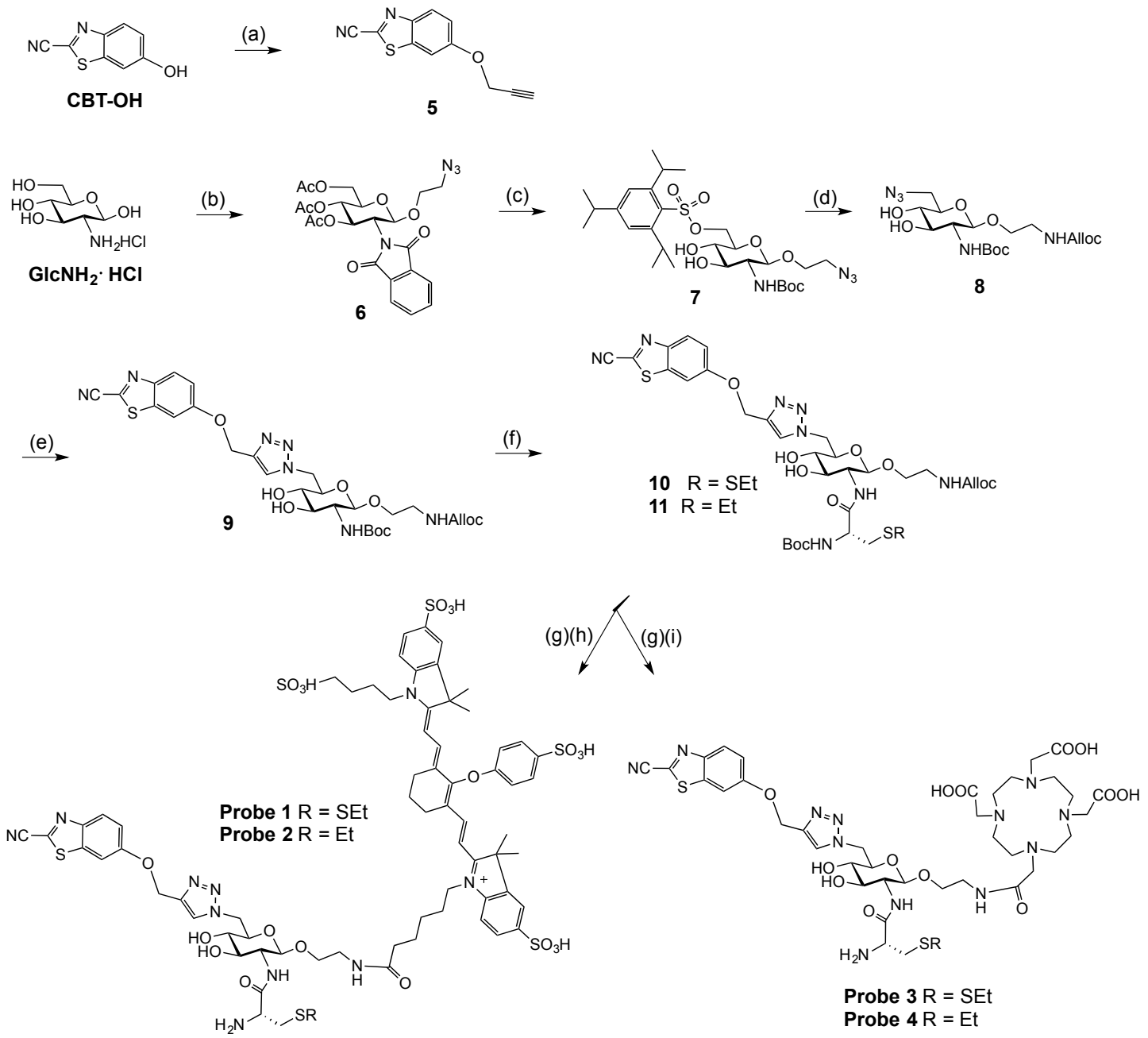


Figure S1. Synthetic scheme of molecular probes 1-4. Reagents and conditions: (a) 2-cyano-6-hydroxybenzothiazole (CBT-OH), propargyl bromide, potassium carbonate, 91%; (b) i. glucosamine hydrochloride, phthalic anhydride, then acetic anhydride/pyridine, 71%; ii. 2-azidoethanol, trimethylsilyl trifluoromethanesulfonate, 0 °C, 86%; (c) i. hydrazine hydrate, 70 °C; ii. di-tert-butyl dicarbonate; iii. 2,4,6-triisopropylbenzenesulfonyl chloride/pyridine, 59% (three steps); (d) i. triphenylphosphine, then allyl chloroformate/pyridine, 73% (two steps); ii. sodium azide, 60 °C, 80%; (e) **5** and **8**, diisopropylethylamine (DIPEA), copper iodide I, Ar, r.t., 77%; (f) 20% TFA, then Boc-Cys(SEt)-OH•DCHA (or Boc-Cys(Et)-OH for compound **11**), HBTU, HOBt, DIPEA, 91% for compound **10** (95% for compound **11**); (g) phenylsilane, tetrakis(triphenylphosphine)palladium(0); (h) IRDye 800CW-NHS ester, DIPEA, then 20% TFA, yield 88% for compound **1** and 85% for compound **2**; (i) DOTA-NHS ester, DIPEA, then 20% TFA, yield 92% for compound **3** and 96% for compound **4**.

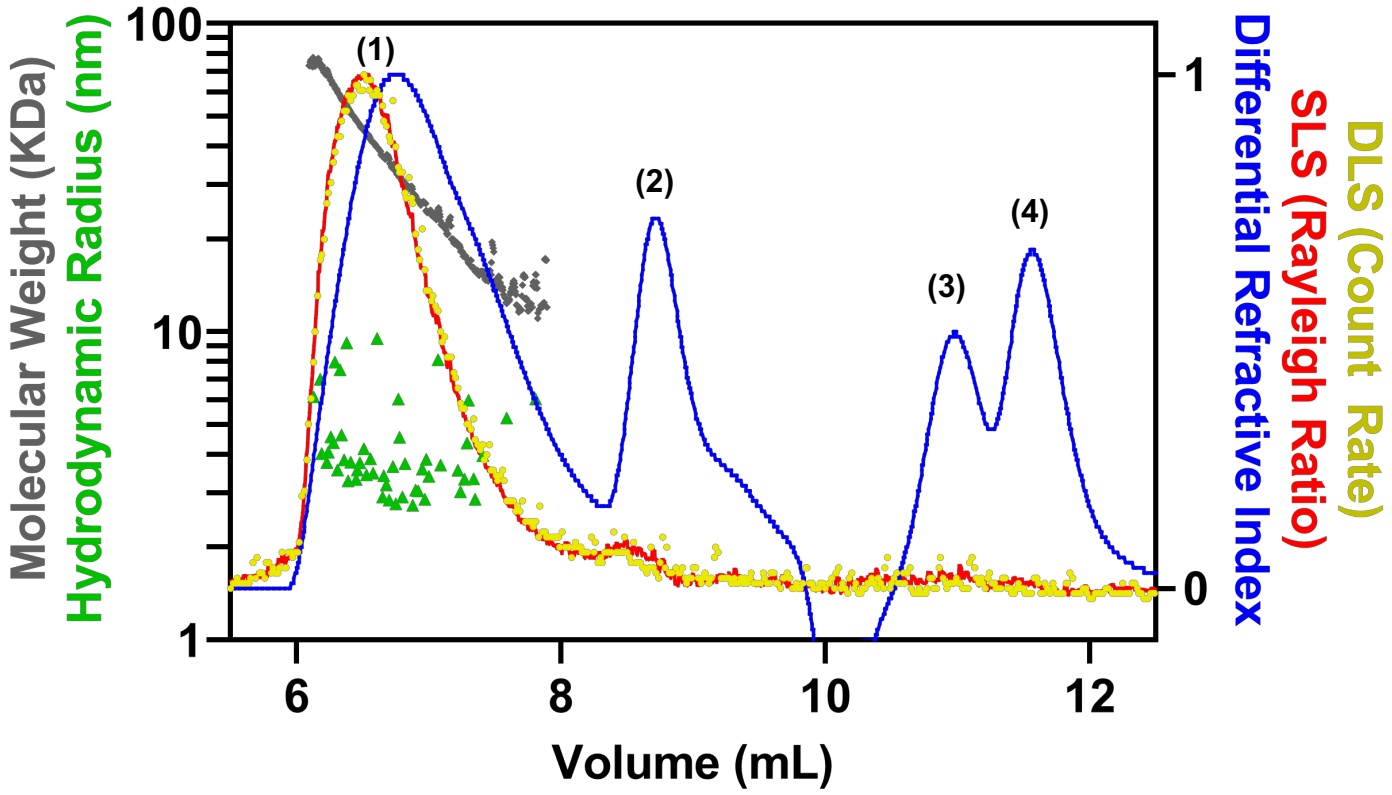


Figure S2. SEC-MALS of probe 3 polymerization at 10 mM. Size exclusion chromatography (WTC010S5 column) coupled to multiangle light scattering (SEC-MALS) was used to determine molecular weight (grey) and hydrodynamic radius (green) of polymerized probe 3. Static (red) and dynamic (yellow) light scattering were measured by a HELEOS instrument (Wyatt Technology) equipped with a Quasi Elastic Light Scattering (QELS) detector, while differential refractometry index (blue) was measured with an Optilab instrument (Wyatt Technology). The elution profile was also detected using a diode array UV-Vis detector (Waters, the UV traces were not shown due to saturation of the signal). Peak (1), 40 kDa average; peak (2), 20 kDa; peak (3), oligomers; peak (4), monomers (activated and unactivated) and dimers (linear and cyclic).

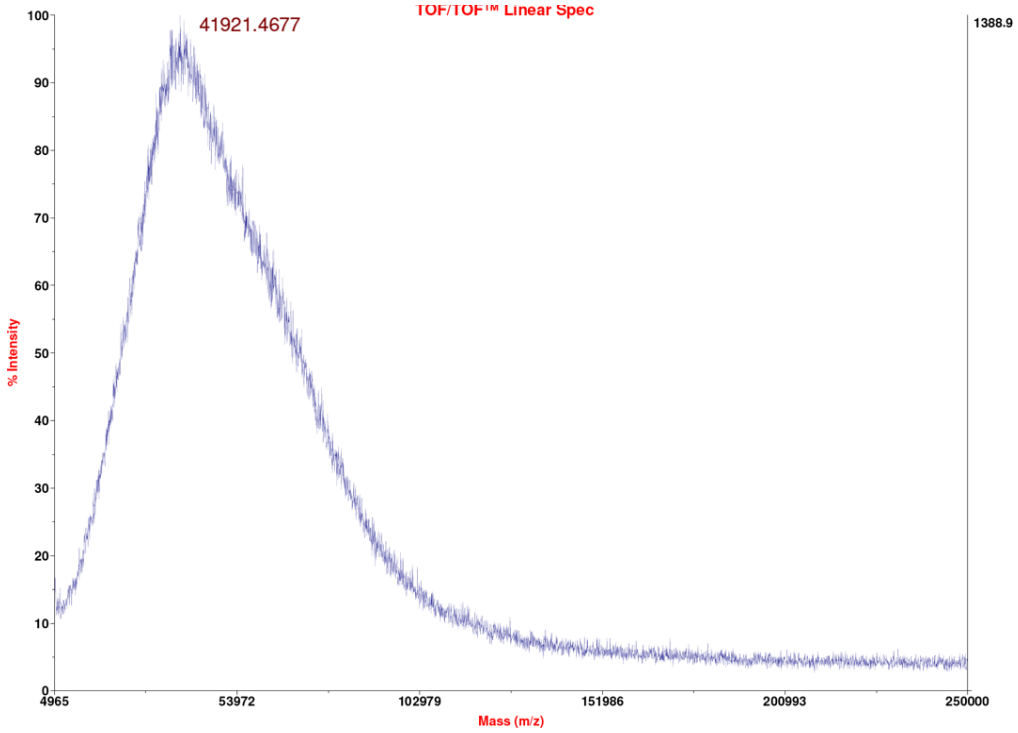


Figure S3. Mass confirmation of peak 1 from S2 using MALDI-TOF mass analysis. Sample from SEC was desalted using ZipTip (C18, P10, Millipore), and the mass was acquired using CHCA matrix in a linear mode on an AB SCIEX 5800 TOF/TOF System.

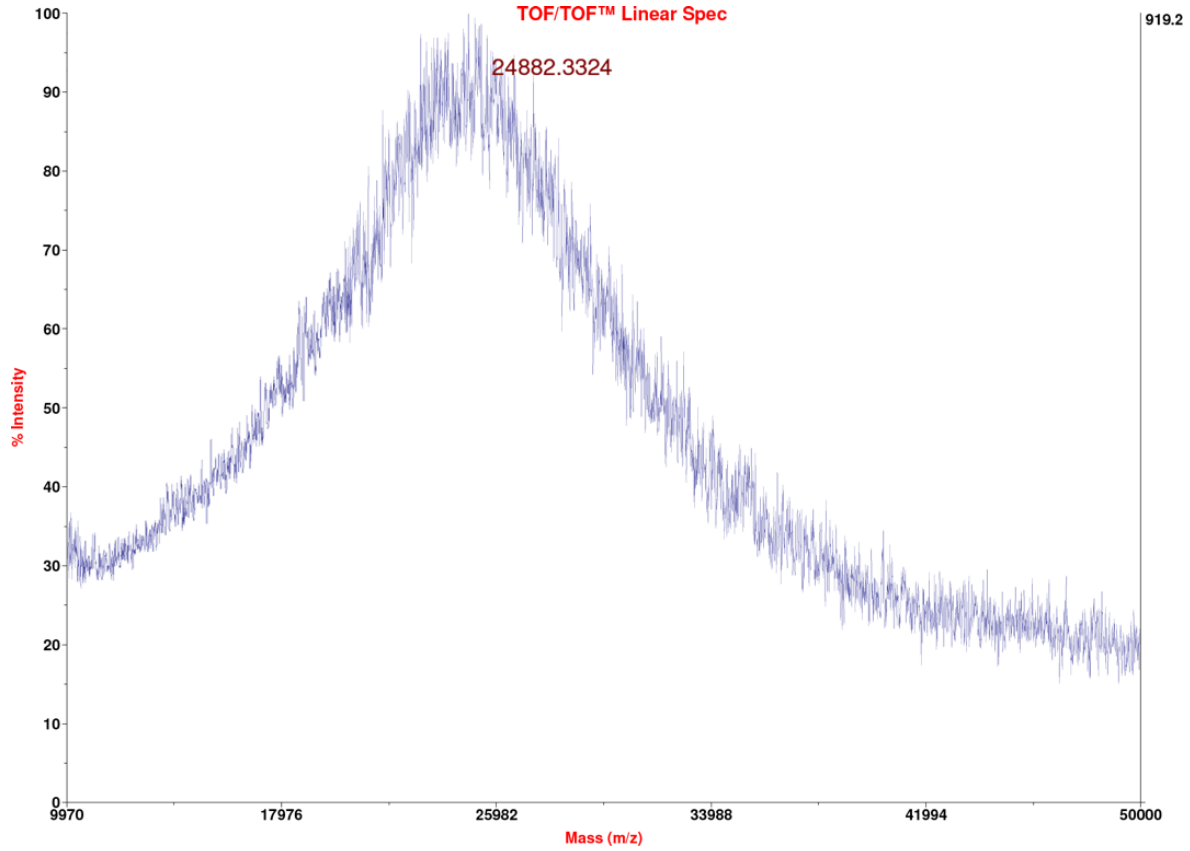


Figure S4. Mass confirmation of peak 2 from S2 using MALDI-TOF mass analysis. Sample from SEC was desalted using ZipTip (C18, P10, Millipore), and the mass was acquired using CHCA matrix in a linear mode on an AB SCIEX 5800 TOF/TOF System.

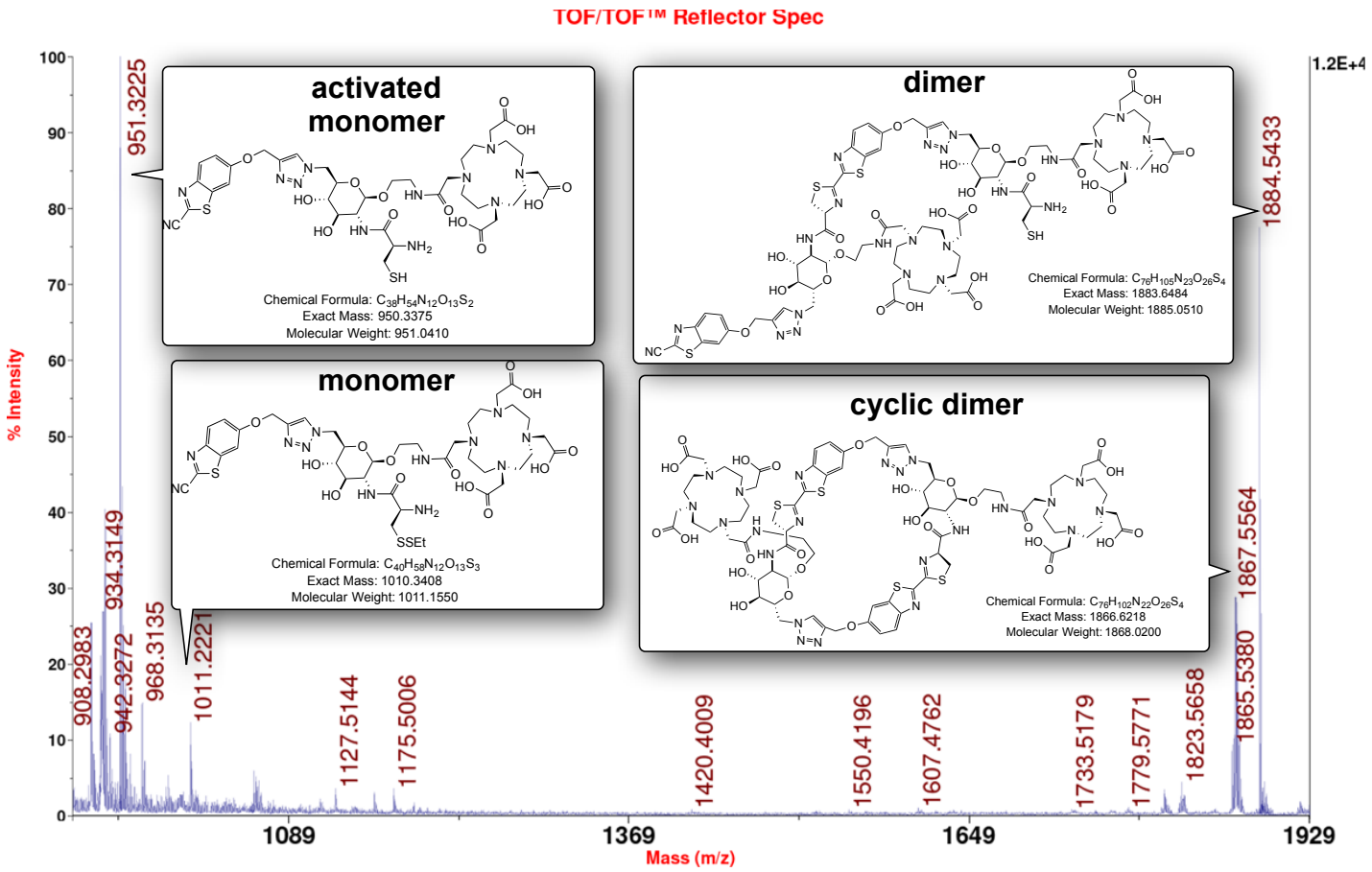


Figure S5. Mass confirmation of peak 4 from S2 using MALDI-TOF mass analysis. Sample from SEC was desalted using ZipTip (C18, P10, Millipore), and the mass was acquired using CHCA matrix in a reflective mode on an AB SCIEX 5800 TOF/TOF System. Unreacted monomer **3**, activated monomer, linear dimer, and cyclic dimer were all detected.

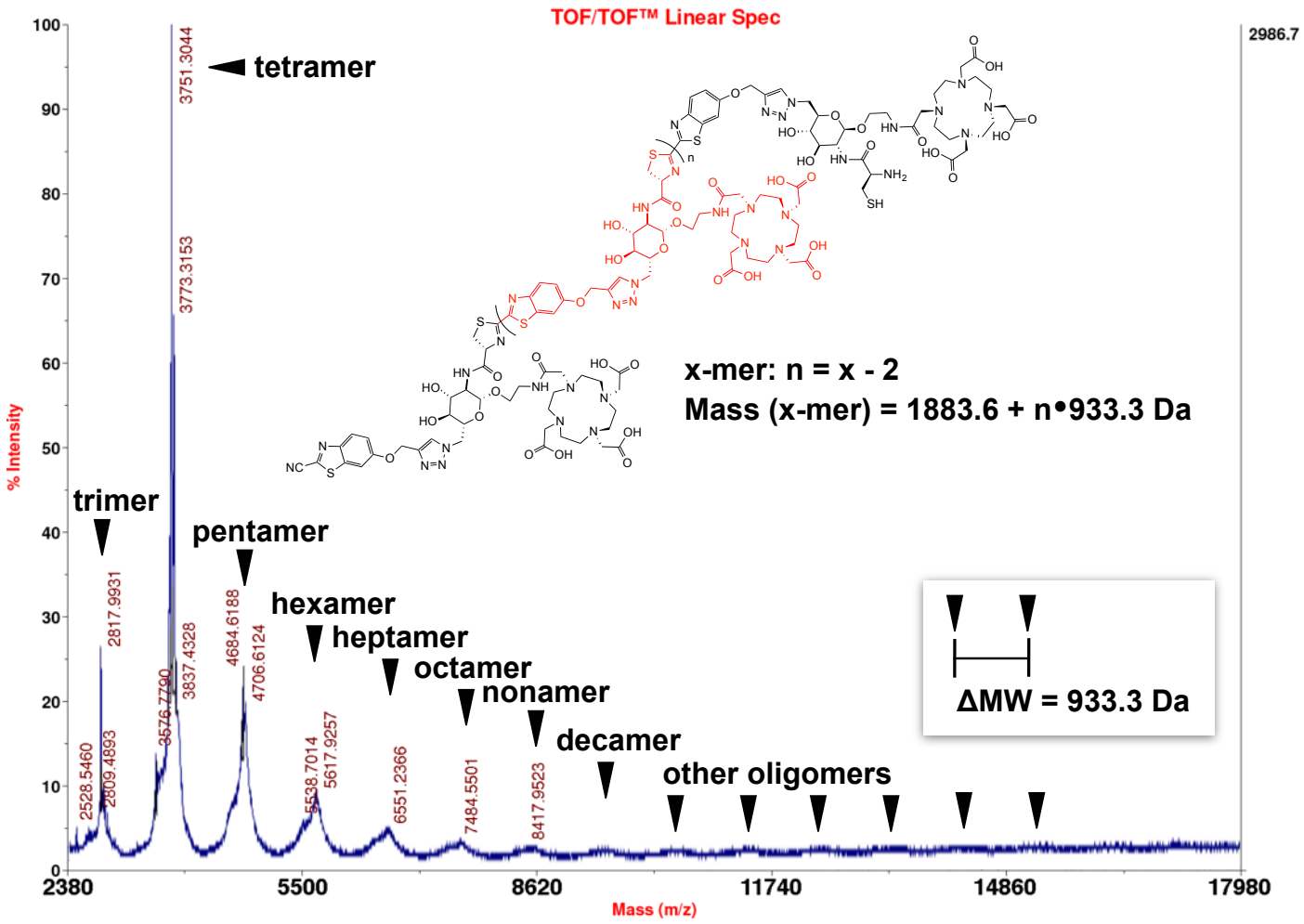


Figure S6. Mass confirmation of peak 3 from S2 using MALDI-TOF mass analysis. Sample from SEC was desalted using ZipTip (C18, P10, Millipore), and the mass was acquired using CHCA matrix in a linear mode on an AB SCIEX 5800 TOF/TOF System. Oligomers were detected ranging from trimer up to species larger than decamer.

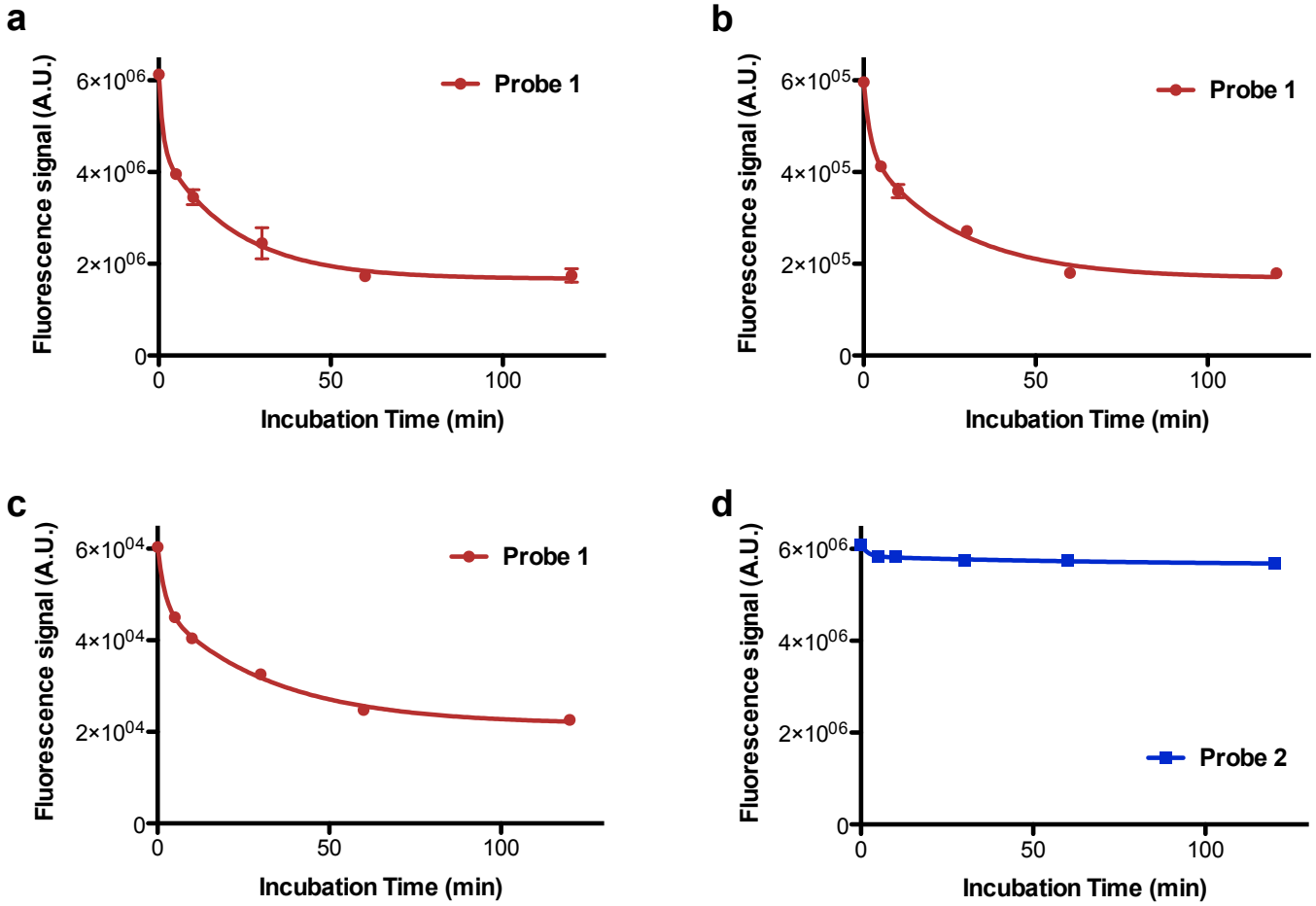


Figure S7. Characterization of polymerization using probe fluorescence (continued). Fluorescence intensity (from fluorometer) of probe **1** at 0.1 μM (**a**), 1 μM (**b**), 10 μM (**c**) and probe **2** at 10 μM (**d**) vs. time in PBS buffer containing GSH (1 mM) at pH 7.4.

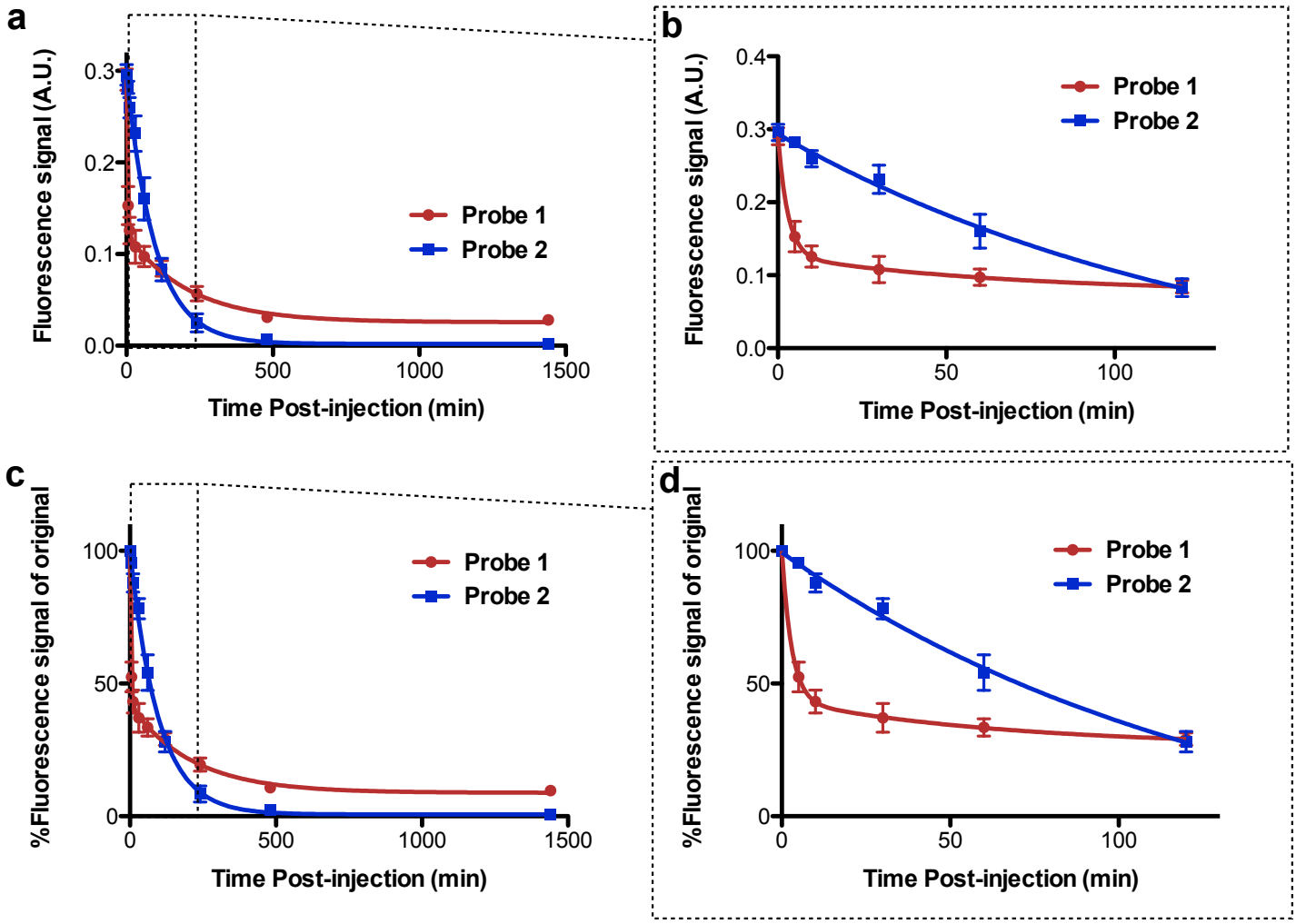


Figure S8. Fluorescence intensity of subcutaneously injected probe 1 and probe 2. a, Fluorescence signal of spots subcutaneously (pre-treated with GSH) injected of probe **1** and probe **2** over 24 hours. **b**, The first two hours of graph **a**. **c**, Percentage of the initial fluorescence signal of subcutaneous probe **1** and **2**. **d**, The first two hours of graph **c**.

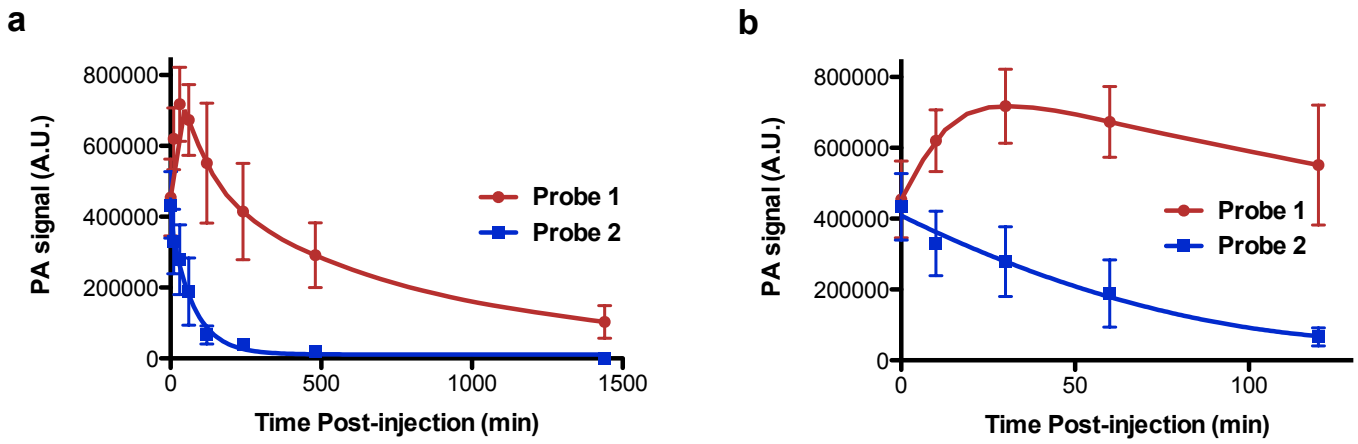


Figure S9. Photoacoustic intensity of subcutaneously injected probe 1 and probe 2. a, Photoacoustic intensity (acquired using Vevo LAZR, quantified using imageJ) of subcutaneously (at spots pre-treated with GSH) injected probe **1** (1 nmol, red) and probe **2** (1 nmol, blue) over 24 hours. **b,** The first two hours of graph **a.**

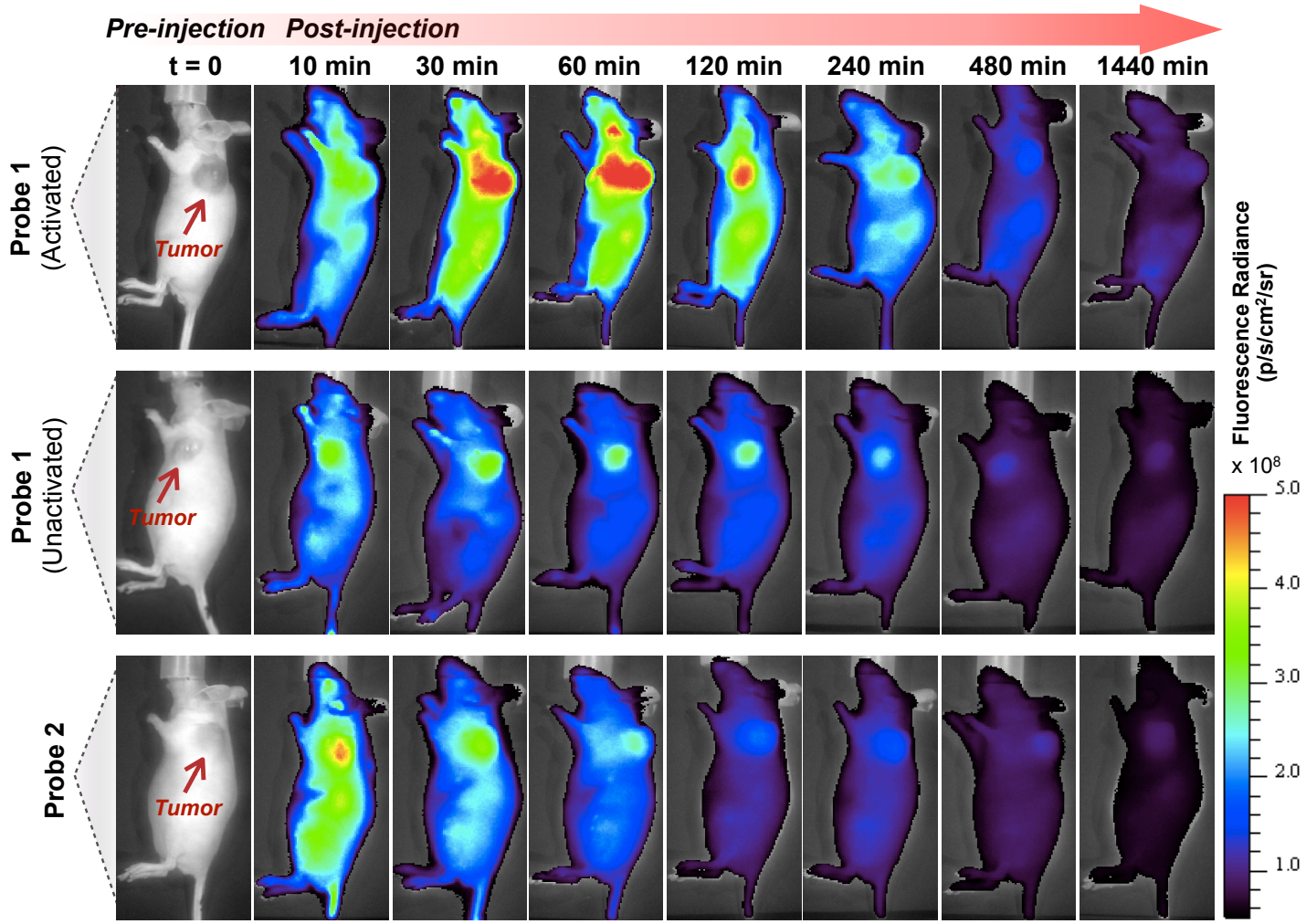


Figure S10. Fluorescence imaging of polymerizable and control probes in tumor models. Comparison of longitudinal fluorescence images of mice with pre-treated tumor (saline or GSH) before and after tail vein injection of probe **1** (20 nmol) or **2** (20 nmol). Anatomical position of the tumor is indicated by red arrow. Probe **1**-activated (20 nmol, GSH pre-treated tumor), probe **1**-unactivated (20 nmol, saline pre-treated tumor), probe **2** (20 nmol, GSH pre-treated tumor).

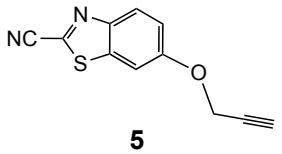
Methods and characterization of compounds

General information

All chemicals were purchased from commercial sources (Aldrich, TCI America, etc) and used without further purification unless otherwise noted. Analytical TLC was performed with 0.25 mm silica gel 60F plates with fluorescent indicator (254 nm). Plates were visualized by ultraviolet light and stained with sulfuric acid (5% aqueous solution, for carbohydrate molecules), nihydrin (1% ethanolic solution, for amino group containing compounds), triphenylphosphine (1% acetonic solution, for organic azides), or potassium permanganate solution. High-performance liquid chromatography (HPLC) was performed on a Dionex HPLC System (Dionex Corporation) equipped with a GP50 gradient pump and an in-line diode array UV-Vis detector. A reversed-phase C18 (Phenomenax, 5 μm, 4.6 x 250 mm or Dionex, 5 μm, 21.2 x 250 mm) column was used with a MeCN/H_2O gradient mobile phase containing 0.1% trifluoroacetic acid (at a flow rate of 1 or 12 mL/min for analysis or purification respectively). High-resolution mass spectrometry was performed on Thermo Orbitrap Elite Velos Pro Detector (Canary Center at Stanford for Cancer Early Detection) and AB SCIEX 5800 TOF/TOF System (Canary Center at Stanford for Cancer Early Detection, reflective or linear mode). MALDI samples were prepared using ZipTip (C18, P10, Millipore), and loaded to plate with matrix α-cyano-4-hydroxycinnamic acid (CHCA) or sinapinic acid (SPA). The ^{1}H and ^{13}C NMR spectra were acquired on Varian 400 MHz (Department of Chemistry, Stanford University) or Bruker 500 MHz or 600 MHz (Department of Chemistry, UC-Berkeley) magnetic resonance spectrometer. Data for ^{1}H NMR spectra are reported as follows: chemical shifts δ are in units of parts per million (ppm) relative to chloroform-d (δ 7.26, s); multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), or br (broadened); coupling constants *J* values are recorded in Hertz (Hz); the number of protons (n) for a given resonance is indicated as nH based on the spectral integration values. Fluorescence imaging was performed on IVIS Spectrum Pre-clinical In Vivo Imaging System (PerkinElmer, MA, USA) or Maestro hyperspectral fluorescent imaging system (PerkinElmer, MA, USA) using a 635±25 nm excitation filter and a 675 nm long-pass emission filter, with images acquired from 670 to 900 nm. Photoacoustic imaging was performed using a Vevo LAZR photoacoustic imaging system, equipped with a

LAZRTight™ imaging enclosure (VisualSonics, Inc.). Animal care and euthanasia were done with the approval of the Administrative Panels on Laboratory Animal Care of Stanford University. All imaging studies were performed at the SCi[3] Core Facility at Stanford.

S11.2. Synthetic procedure and characterization.



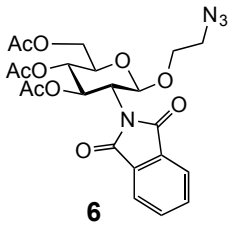
5

2-Cyano-6-propargoxybenzothiazole (5). The precursor 2-cyano-6-hydroxybenzothiazole (CBT-OH) used to prepare compound **5** was made according to previously reported method.[1] Briefly, a mixture of 2-cyano-6-methoxybenzothiazole (500 mg, 2.6 mmol) and pyridine hydrochloride (3.0 g, 26.0 mmol) was heated to 200 °C to liquefy, and the brownish reaction solution was stirred for 1-2 hours until completion as monitored by TLC. The mixture was cooled down to 0 °C and sodium bicarbonate (saturated) was added portion-wise to neutralize the reaction mixture to obtain a yellow suspension. The yellow precipitate was collected by filtration, washed with water, and dried under vacuum. Column chromatography (methanol:dichloromethane, 1:99) gave the product as a yellow powder (340 mg, yield 74%). To a solution of CBT-OH (370 mg, 2.0 mmol) in anhydrous acetonitrile (3 mL) was added propargyl bromide (936 μL, 10.5 mmol) and potassium carbonate (871 mg, 6.3 mmol). The reaction mixture was stirred overnight at r.t. and extracted by ammonium chloride/dichloromethane. The organic layer was dried over anhydrous sodium sulfate, and concentrated. Column chromatography (pure dichloromethane) gave the product as a pale yellow powder (390 mg, yield 91%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 8.19 (d, J = 9.1 Hz, 1H, Ar), 7.96 (s, 1H, Ar), 7.39 (d, J = 9.1 Hz, 1H, Ar), 4.96 (d, J = 2.2 Hz, 2H, CH_2O), 3.66 (t, J = 2.2 Hz, 1H, alkyne).

^{13}C NMR (75 MHz, DMSO-d_6): δ 158.1, 147.1, 137.8, 134.7, 125.8, 119.2, 114.0, 106.6, 79.3 (alkyne), 78.9 (alkyne), 56.7 (CH_2O).

HRMS (ESI) calc'd for $C_{11}H_7N_2OS^+$ ($M+H^+$): 215.0279, found: 215.0272.



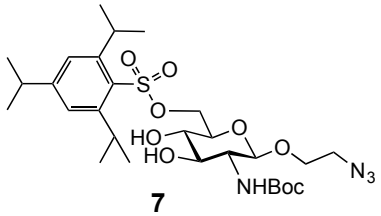
6

2-Azidoethyl 2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (6). To a solution of D-glucosamine hydrochloride (50.0 g, 232 mmol) in water (500 mL) was added sodium bicarbonate (97.6 g, 1.16 mol) and phthalic anhydride (68.7 g, 464 mmol). The reaction mixture was stirred overnight at r.t. and was evaporated to dryness. The white residue containing *N*-phthalimido protected glucosamine was then redissolved in a mixture of acetic anhydride (500 mL) and pyridine (500 mL) and stirred at r.t. for 48 hours until all hydroxyl groups were acetylated (monitored by TLC). The reaction mixture was then treated with ice-cold water, and the resultant precipitate was collected by filtration to give the 2-deoxy-2-phthalimido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside as a white powder (78.6 g, 71%). Without further purification, the product (10.0 g, 20.9 mmol) was stirred in anhydrous dichloromethane (100 mL) with freshly made 2-azidoethanol (1.8 g, 20.9 mmol) with activated molecular sieves (4 Å) at r.t. for 1 hour. The reaction mixture was then cooled to 0 °C and trimethylsilyl trifluoromethanesulfonate (1.9 mL, 10.5 mmol) was added dropwise over 5 min. The reaction was stirred at 0 °C for 8 hours and quenched by the addition of triethylamine until neutral. The solvents were then removed and column chromatography (ethyl acetate:hexanes, 1:3) gave the product as a white powder (9.1 g, 86%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (m, 4H, Ar), 5.46 (dd, J = 10.0, 9.8 Hz, 1H, H-3), 5.18 (d, 1H, J = 8.5 Hz, H-1), 5.15 (dd, J = 9.8, 9.8 Hz, 1H, H-4), 4.4 (dd, J = 10.0, 8.5 Hz, 1H, H-2), 4.3 (dd, J = 12.6, 4.9 Hz, 1H, H-6a), 4.1 (dd, J = 12.6, 2.2 Hz, 1H, H-6b), 4.00 (m, 1H, H-5), 3.61 (t, 2H, J = 5.1 Hz, CH_2CH$_2$N$_3$), 3.37 (t, 2H, J = 5.1 Hz, CH$_2$N$_3$), 2.05 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.93 (s, 3H, OAc).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0 (OCOCH$_3$), 170.7 (OCOCH$_3$), 170.5 (OCOCH$_3$), 167.6 (Ar-CO), 167.3 (Ar-CO), 133.0 (Ar), 132.9 (Ar), 132.5 (Ar), 132.4 (Ar), 124.1 (Ar), 124.0 (Ar), 102.3 (C-1), 74.3, 70.9, 70.8 (C-3, C-4, C-5), 63.8, 62.9, 62.5 (C-2, C-6, CH$_2$CH$_2$N$_3$), 50.5 (CH$_2$N$_3$), 20.3 (OCOCH$_3$), 20.2 (OCOCH$_3$), 20.0 (OCOCH$_3$).

HRMS (ESI) calc'd for $C_{22}H_{24}N_4NaO_{10}^+$ (M+Na$^+$): 527.1390, found: 527.1389.



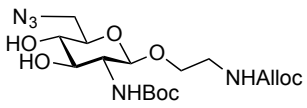
7

2-Azidoethyl 2-(*N-tert*-butoxycarbonylamino)-2-deoxy-6-*O*-(2,4,6-triisopropylbenzenesulfonyl)-β-D-glucopyranoside (7). Compound **6** (10.6 g, 21.0 mmol) was dissolved in ethanol (80 mL), followed by the addition of hydrazine hydrate (24 mL). The reaction was heated to 70 °C for 30 min until all protecting groups were removed (monitored by TLC), solvents were removed by rotary evaporation, and the residue was dried under vacuum. The crude product was then all dissolved in water-THF (100 mL each) containing sodium bicarbonate (18.0 g, 0.21 mol). Di-tert-butyl dicarbonate [$(Boc)_2O$, 19.5 mL, 84.9 mmol] was added portion-wise over 30 min at 0 °C, and the biphasic reaction mixture was stirred at r.t. overnight until all amino groups were consumed (monitored by TLC). The solvents were removed and the dried residue was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (19.3 g, 63.7 mmol) in dry pyridine (100 mL) at r.t. overnight. Reaction mixture was then evaporated to dryness and column chromatography (methanol:dichloromethane) gave the compound **7** as a white powder (7.6 g, 59% over three steps).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.19 (s, 2H, Ar), 4.50 (d, 1H, J = 7.9 Hz, H-1), 4.40 (dd, J = 10.9, 1.9 Hz, 1H, H-6a), 4.25 (dd, J = 10.3, 6.8 Hz, 1H, H-6b), 4.13 (dd, J = 13.5, 6.7 Hz, 1H, H-2), 3.98 (m, 1H, H-5), 3.76 – 3.56 (m, 4H, H-3, H-4, $CH_2CH_2N_3$), 3.47 – 3.37 (m, 2H, CH_2N_3), 2.92 (m, 3H, $(CH_3)_2CH$), 1.51 – 1.45 (s, 9H, $(CH_3)_3C$), 1.25 (d, J = 12.4 Hz, 18H, $(CH_3)_2CH$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 157.1 (Ar), 153.7(Ar), 150.8 (Ar), 129.2 (Ar), 127.1 (Ar), 123.7 (Ar), 103.5 (C-1), 81.9 ($(CH_3)_3C$), 80.6, 77.4, 77.0, 76.6, 73.8, 68.2 (C-3, C-4, C-5, C-2, C-6, $CH_2CH_2N_3$), 50.7 (CH_2N_3), 34.2 ($(CH_3)_2CH$), 29.6 ($(CH_3)_3C$), 24.7 ($(CH_3)_2CH$).

HRMS (ESI) calc'd for $C_{28}H_{46}N_4NaO_9S^+$ ($M+Na^+$): 637.2883, found: 637.2882.



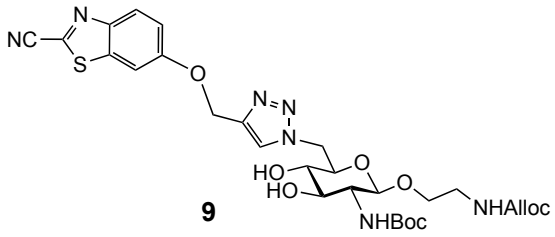
8

***2-(N*-Allyloxycarbonylamino)ethyl 6-azido-2-(*N-tert*-butoxycarbonylamino)-2,6-*di*-deoxy-β-D-glucopyranoside (8).** Compound **7** (3.0 g, 4.9 mmol) was dissolved in a mixture of methanol-dichloromethane (100 mL each) containing triphenylphosphine (1.9 g, 7.4 mmol), and stirred for overnight at r.t. until azido groups were reduced to free amines (monitored by TLC). The solvents were then removed and the solid residue was treated with allyl chloroformate (1.0 mL, 9.8 mmol) in anhydrous pyridine/dichloromethane (3 mL/10 mL) at r.t. overnight. The solvents were then removed and column chromatography (methanol:dichloromethane, 2:98) gave the product as a white powder (2.4 g, 73% over two steps), which (1.6 g, 2.38 mmol) was dissolved in dry DMF (10 mL), followed by the addition of sodium azide (1.5 g, 23.8 mmol). The reaction mixture was heated at 60 °C for 24 hours, and was extracted with ethyl acetate/water (100 mL each). The organic layer was dried and concentrated, and column chromatography (methanol:dichloromethane 1:99 to 3:97) gave compound **8** as a white powder (821 mg, 80%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.94 (ddd, J = 22.7, 10.8, 5.7 Hz, 1H, alkene), 5.40 – 5.18 (m, 2H, alkene), 4.71 – 4.42 (m, 3H, H-1, alkene-CH_2), 3.98 – 3.26 (m, 12H, H-2, H-3, H-4, H-5, H-6a, H-6b, CH_2N_3, OCH_2CH$_2$, NHCH_2CH$_2$), 1.55 – 1.42 (m, 9H, $(CH_3)_3$C).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (C=O), 157.3 (C=O), 133.02 (alkene), 116.1 (alkene), 101.8 (C-1), 76.1 ($(CH_3)_3C$), 75.5, 74.2, 72.3, 71.5, 71.2, 68.1, 56.0 (C-2, C-3, C-4, C-5, C-6, NHCH$_2$CH$_2$O, alkene-CH$_2$), 50.9 (CH_2N_3), 40.4 (NHCH$_2$), 27.4 ($(CH_3)_3$C).

HRMS (ESI) calc'd for $C_{17}H_{29}N_5NaO_8^+$ (M+Na$^+$): 454.1914, found: 454.1902.



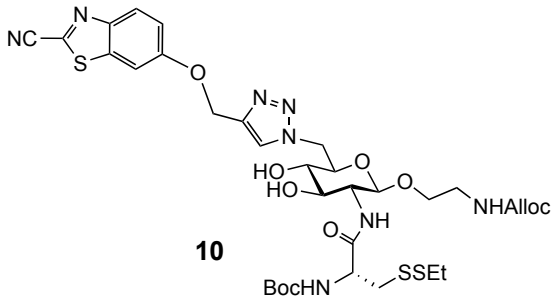
9

Compound 9. Compounds **5** (109 mg, 0.51 mmol) and **8** (200 mg, 0.46 mmol) were dissolved in THF (4 mL) containing N,N-diisopropylethylamine (DIPEA) (242 μL, 1.39 mmol). The reaction mixture was degassed using argon, and copper iodide I (8.8 mg, 0.051 mmol) was added under argon protection. The reaction mixture was stirred overnight at r.t. and purified using HPLC (50% acetonitrile/water to 100% acetonitrile, with 0.1% TFA, 12 mL/min, 30 min). Product **9** was obtained as a white-light-yellow powder after lyophilization (231 mg, 77%).

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.20 (s, 1H, triazole-H), 8.17 (d, J = 9.1 Hz, 1H), 8.04 (d, J = 2.5 Hz, 1H, Ar), 7.38 (dd, J = 9.1, 2.5 Hz, 1H, Ar), 6.91 (s, 1H, NH), 6.67 (d, J = 7.5 Hz, 1H, NH), 5.84 (ddd, J = 17.0, 10.7, 5.2 Hz, 1H, alkene), 5.47 (d, J = 5.5 Hz, 1H, OH), 5.30 (s, 2H, triazole-CH_2), 5.27 – 5.01 (m, 2H, alkene, OH), 4.78 (d, J = 14.0 Hz, 1H, NH), 4.60 – 4.33 (m, 2H, alkene-CH_2), 4.20 (d, J = 8.2 Hz, 1H, H-1), 3.68 – 2.93 (m, 10H, H-2, H-3, H-4, H-5, H-6a, H-6b, NHCH_2CH_2), 1.37 (s, 9H, $(CH_3)_3$C).

^{13}C NMR (125 MHz, DMSO-d_6): δ 159.1 (Ar-*C*-O), 156.3 (C=O), 155.9 (C=O), 146.8 (Ar), 142.2 (Ar), 137.9 (Ar), 134.3 (Ar), 134.05, 126.36, 125.8, 119.3, 117.5, 114.0, 105.9 (Ar, alkene, alkyne, triazole), 101.9 (C-1), 77.9 ($(CH_3)_3C$), 74.8, 74.1, 72.2, 67.5, 64.8, 62.3 (C-2, C-3, C-4, C-5, triazole-CH_2, NHCH_2CH_2), 56.9 (C-6), 41.0 (NHCH_2), 28.7 ($(CH_3)_3$C).

HRMS (ESI) calc'd for $C_{28}H_{35}N_7NaO_9S^+$ (M+Na^+): 668.2115, found: 668.2110.



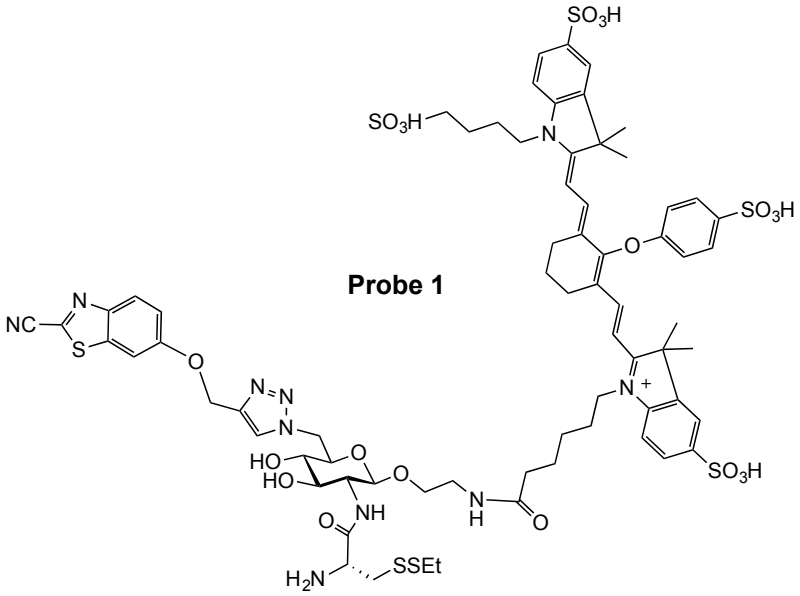
10

Compound 10. Compound **9** (200 mg, 0.31 mmol) was dissolved in TFA-dichloromethane solution (2 mL, containing 20% TFA, 75% dichloromethane, 4% acetonitrile, 1% triisopropylsilane) at r.t. for 1 hour until complete removal of Boc protecting group (monitored by HPLC). The reaction mixture was evaporated to dryness and the deprotected product was used directly in the next step reaction. To a solution of deprotected compound (100 mg, 0.18 mmol) and Boc-Cys(SEt)-OH•DCHA (102 mg, 0.22 mmol, Bachem) in dry DMF (2 mL) were added HBTU (102 mg, 0.27 mmol), HOBt (41 mg, 0.27 mmol). DIPEA (> 94 μL or 0.54 mmol) was added until pH around 7 to 8. The reaction was completed at r.t. over 1 hour (monitored by HPLC), and the mixture was purified by HPLC to give product as a white powder after lyophilization (132 mg, 91%).

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.21 (s, 1H, triazole-H), 8.17 (d, J = 9.5 Hz, 1H, Ar), 8.03 (d, J = 2.5 Hz, 1H, Ar), 7.80 (d, J = 9.0 Hz, 1H, NH), 7.38 (dd, J = 9.1, 2.5 Hz, 1H, Ar), 6.98 (d, J = 9.1 Hz, 1H, NH), 5.83 (ddd, J = 17.1, 10.5, 5.0 Hz, 1H, alkene), 5.49 (d, J = 5.6 Hz, 1H, OH), 5.30 (s, 2H, triazole-CH_2), 5.21 (ddd, J = 17.6, 1.6, 1.6 Hz, 1H, alkene), 5.12 (ddd, J = 11.1, 1.6, 1.6 Hz, 1H, alkene), 5.02 (d, J = 4.7 Hz, 1H, OH), 4.78 (dd, J = 14.0, 2.0 Hz, 1H, NH), 4.41 (d, J = 4.5 Hz, 2H, alkene-CH_2), 4.34 (d, J = 8.2 Hz, 1H, H-1), 4.16 (m, 1H, Cys-CH), 3.68 – 3.25 (m, 8H, H-2, H-3, H-4, H-5, H-6a, H-6b, NHCH$_2$CH_2O), 3.15 – 2.95 (m, 3H, NHCH_2, SCH_2CH), 2.77 (dd, J = 13.4, 10.9 Hz, 1H, SCH_2CH), 2.74 (q, J = 6.9 Hz, 2H, SCH_2CH$_3$), 1.39 (s, 9H, (CH$_3$)$_3$C), 1.17 (t, J = 7.2 Hz, 3H, SCH$_2$CH_3).

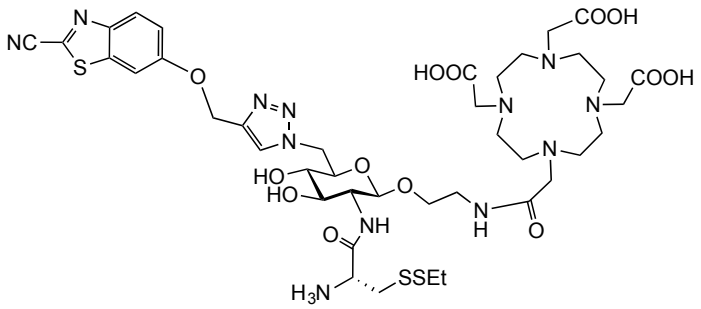
^{13}C NMR (125 MHz, DMSO-d_6): δ 170.7 (Cys-CO), 159.1 (Ar-C-O), 156.4 (C=O), 155.8 (C=O), 146.8 (Ar), 142.3 (Ar), 137.9 (Ar), 134.3 (Ar), 134.1, 126.4, 125.8, 119.3, 117.4, 114.1, 106.0 (Ar, alkene, alkyne, triazole), 101.5 (C-1), 78.8 ((CH$_3$)$_3C$), 74.8, 73.9, 72.1, 68.0, 64.8, 62.3 (C-2, C-3, C-4, C-5, triazole-CH$_2$, NHCH$_2C$H$_2$), 55.8 (C-6), 54.3 (Cys-CH), 51.4, 41.8 (NHCH$_2$), 39.2 (SCH$_2$CH), 32.0 (SCH$_2C$H$_3$), 28.6 ((CH$_3$)$_3$C), 14.7 (SCH$_2C$H$_3$).

HRMS (ESI) calc'd for $C_{33}H_{44}N_8NaO_{10}S_3^+$ ($M+Na^+$): 831.2240, found: 831.2249.



Compound 1. Compound **10** (2 mg, 2.7 μmol) was dissolved in dichloromethane (100 μL) containing phenylsilane (1 μL, 7.8 μmol), and the reaction solution was degassed using argon. Bright yellow crystalline tetrakis(triphenylphosphine)palladium(0) (1.0 mg, 0.87 μmol) was added under argon protection, and the reaction was stirred for 30 min until allyl groups were fully removed (monitored by HPLC). The reaction mixture was then evaporated to dryness and the residue containing free amine was used directly in the next step coupling reactions. Briefly, the crude amine (2.7 μmol) was dissolved in anhydrous DMF (80 μL) and IRDye 800CW-NHS ester (120 μL, 20 mM in DMSO, 2.4 μmol, LI-COR Biosciences), followed by the addition of DIPEA (1.4 μL, 8.1 μmol). The reaction reached completion in 1 hour at r.t. (monitored by HPLC), and the mixture was purified by HPLC. The product collected was lyophilized to dryness and was dissolved in TFA-dichloromethane solution (200 μL, containing 20% TFA, 75% dichloromethane, 4% acetonitrile, 1% triisopropylsilane) at r.t. for 1 hour until complete removal of Boc protecting group (monitored by HPLC). The final product was purified by HPLC, and dried by lyophilization to give compound **1** a deep green powder (3.4 mg, 88%).

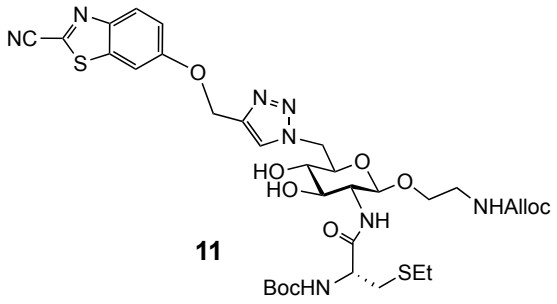
MALDI TOF/TOF HRMS calc'd for $C_{70}H_{85}N_{10}O_{20}S_7^+$ (M^+): 1609.3981, found: 1609.4646.



Probe 3

Compound 3. Compound **10** (30 mg, 0.041 mmol) was dissolved in dichloromethane (200 μL) containing phenylsilane (0.062 mmol, 8 μL), and the reaction solution was degassed using argon. Bright yellow crystalline tetrakis(triphenylphosphine)palladium(0) (4.7 mg, 4.1 μmol) was added under argon protection, and the reaction was stirred for 30 min until allyl groups were fully removed (monitored by HPLC). The reaction mixture was then evaporated to dryness and the residue containing free amine was used directly in the next step coupling reactions. Briefly, the crude free amine (0.041 mmol) and 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-NHS ester) (37 mg, 0.049 mmol, Macrocyclics) were dissolved together in anhydrous DMF (200 μL), and DIPEA (21 μL, 0.12 mmol) was added to adjust the pH. The reaction reached completion in 1 hour at r.t. (monitored by HPLC), and the mixture was purified by HPLC. The product collected was lyophilized to dryness and was dissolved in TFA-dichloromethane solution (200 μL, containing 20% TFA, 75% dichloromethane, 4% acetonitrile, 1% triisopropylsilane) at r.t. for 1 hour until complete removal of Boc protecting group (monitored by HPLC). The final product was purified by HPLC, and dried by lyophilization to give a white powder (38 mg, 92%).

MALDI TOF/TOF HRMS calc'd for $C_{40}H_{59}N_{12}O_{13}S_3^+$ ($M+H^+$): 1011.3487, found: 1011.4196.



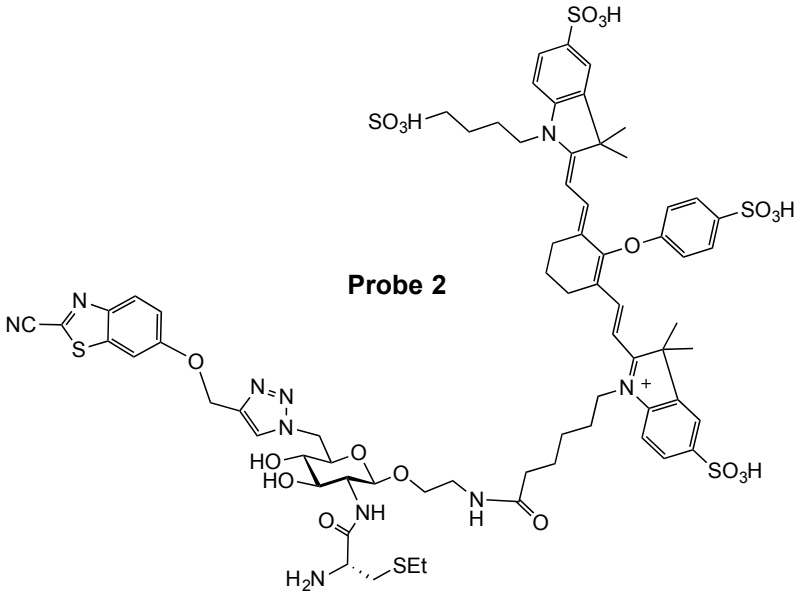
11

Compound 11. Compound **9** (40 mg, 0.062 mmol) was dissolved in TFA-dichloromethane solution (400 μL, containing 20% TFA, 75% dichloromethane, 4% acetonitrile, 1% triisopropylsilane) at r.t. for 1 hour until complete removal of Boc protecting group (monitored by HPLC). The reaction mixture was evaporated to dryness and the free amine was used directly in the next step reaction. To a solution of the free amine (20 mg, 0.036 mmol) and Boc-Cys(Et)-OH (20 mg, 0.044 mmol, Bachem) in dry DMF (400 μL) were added HBTU (20 mg, 0.054 mmol), HOBt (8.2 mg, 0.054 mmol). DIPEA (> 19 μL or 0.11 mmol) was added until pH around 7 to 8. The reaction was completed at r.t. over 1 hour (monitored by HPLC), and the mixture was purified by HPLC to give product **11** as a white powder after lyophilization (27 mg, 95%).

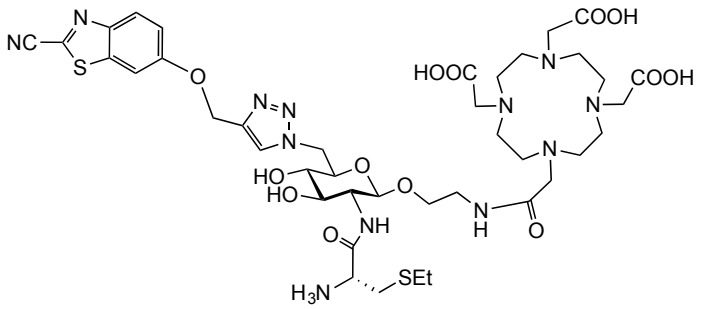
^{1}H NMR (500 MHz, DMSO-d_6): δ 8.21 (s, 1H, triazole-H), 8.16 (d, J = 9.5 Hz, 1H, Ar), 8.04 (d, J = 2.5 Hz, 1H, Ar), 7.83 (d, J = 7.0 Hz, 1H, NH), 7.38 (dd, J = 9.1, 2.5 Hz, 1H, Ar), 6.80 (d, J = 8.9 Hz, 1H, NH), 5.83 (ddd, J = 17.0, 10.1, 4.5 Hz, 1H, alkene), 5.50 (d, J = 5.6 Hz, 1H, OH), 5.30 (s, 2H, triazole-CH_2), 5.21 (dd, J = 17.0, 1.6 Hz, 1H, alkene), 5.12 (dd, J = 10.1, 1.2 Hz, 1H, alkene), 5.01 (d, J = 4.0 Hz, 1H, OH), 4.78 (dd, J = 14.0, 2.0 Hz, 1H, NH), 4.60 – 4.39 (m, 2H, alkene-CH_2), 4.33 (d, J = 8.2 Hz, 1H, H-1), 3.99 (m, 1H, Cys-CH), 3.55–3.30 (m, 8H, H-2, H-3, H-4, H-5, H-6a, H-6b, NHCH$_2$CH_2O), 3.20 – 2.95 (m, 2H, NHCH_2), 2.85 (dd, J = 13.4, 10.9 Hz, 1H, SCH_2CH), 2.65 – 2.50 (m, 3H, SCH_2CH, SCH_2CH$_3$), 1.42 (s, 9H, (CH$_3$)$_3$C), 1.18 (t, J = 7.2 Hz, 3H, SCH$_2$CH_3).

^{13}C NMR (125 MHz, DMSO-d_6): δ 171.0 (Cys-CO), 159.1 (Ar-C-O), 156.4 (C=O), 155.8 (C=O), 146.8 (Ar), 137.9 (Ar), 134.3 (Ar), 134.1, 126.4, 125.8, 119.3, 117.4, 114.1, 106.0 (Ar, alkene, alkyne, triazole), 101.4 (C-1), 78.7 ((CH$_3$)$_3$$C$), 74.8, 74.0, 72.1, 68.0, 64.8, 62.3 (C-2, C-3, C-4, C-5, triazole-CH$_2$, NHCH$_2$$CH_2$), 55.9 (C-6), 54.9 (Cys-CH), 51.4, 34.2 (SCH$_2$CH), 28.6 ((CH$_3$)$_3$C), 25.6 (SCH$_2$$CH_3$), 15.2 (SCH$_2$$CH_3$).

HRMS (ESI) calc'd for $C_{33}H_{44}N_8NaO_{10}S_2$ (M+Na$^+$): 799.2520, found: 799.2525.



Compound 2. Compound **11** (1 mg, 1.4 μmol) was dissolved in dichloromethane (100 μL) containing phenylsilane (1 μL, 7.8 μmol), and the reaction solution was degassed using argon. Bright yellow crystalline tetrakis(triphenylphosphine)palladium(0) (1.0 mg, 0.87 μmol) was added under argon protection, and the reaction was stirred for 30 min until allyl groups were fully removed (monitored by HPLC). The reaction mixture was then evaporated to dryness and the residue containing free amine was used directly in the next step coupling reactions. Briefly, the crude free amine (1.4 μmol) was dissolved in anhydrous DMF (135 μL) and IRDye 800CW-NHS ester (65 μL, 20 mM in DMSO, 1.3 μmol, LI-COR Biosciences), followed by the addition of DIPEA (1.4 μL, 5.8 μmol). The reaction reached completion in 1 hour at r.t. (monitored by HPLC), and the mixture was purified by HPLC. The product collected was lyophilized to dryness and was dissolved in TFA-dichloromethane solution (200 μL, containing 20% TFA, 75% dichloromethane, 4% acetonitrile, 1% triisopropylsilane) at r.t. for 1 hour until complete removal of Boc protecting group (monitored by HPLC). The final product was purified by HPLC, and dried by lyophilization to give a deep green powder (1.7 mg, 85%). **MALDI TOF/TOF HRMS** calc'd for $C_{70}H_{85}N_{10}O_{20}S_6^+$ (M^+): 1577.4260, found: 1577.5229.



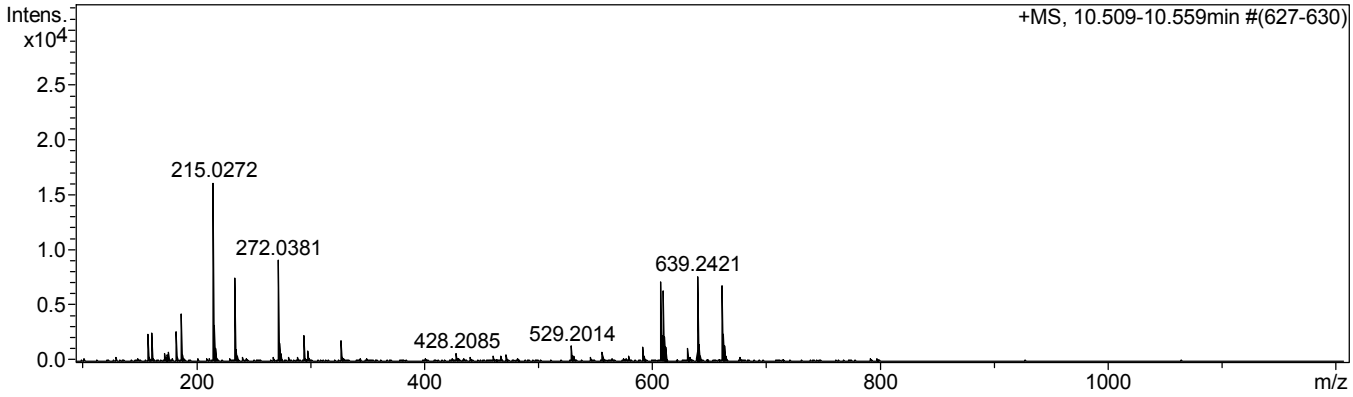
Probe 4

Compound 4. Compound **11** (9.7 mg, 0.014 mmol) was dissolved in dichloromethane (100 μL) containing phenylsilane (0.021 mmol, 2.7 μL), and the reaction solution was degassed using argon. Bright yellow crystalline tetrakis(triphenylphosphine)palladium(0) (1.6 mg, 1.4 μmol) was added under argon protection, and the reaction was stirred for 30 min until allyl groups were fully removed (monitored by HPLC). The reaction mixture was then evaporated to dryness and the residue containing free amine was used directly in the next step coupling reactions. Briefly, the crude free amine (0.014 mmol) and DOTA-NHS ester (12 mg, 0.016 mmol, Macrocyclics) were dissolved together in anhydrous DMF (100 μL), and DIPEA (7 μL, 0.04 mmol) was added to adjust the pH. The reaction reached completion in 1 hour at r.t. (monitored by HPLC), and the mixture was purified by HPLC. The product collected was lyophilized to dryness and was dissolved in TFA-dichloromethane solution (100 μL, containing 20% TFA, 75% dichloromethane, 4% acetonitrile, 1% triisopropylsilane) at r.t. for 1 hour until complete removal of Boc protecting group (monitored by HPLC). The final product was purified by HPLC, and dried by lyophilization to give a white powder (13 mg, 96%).

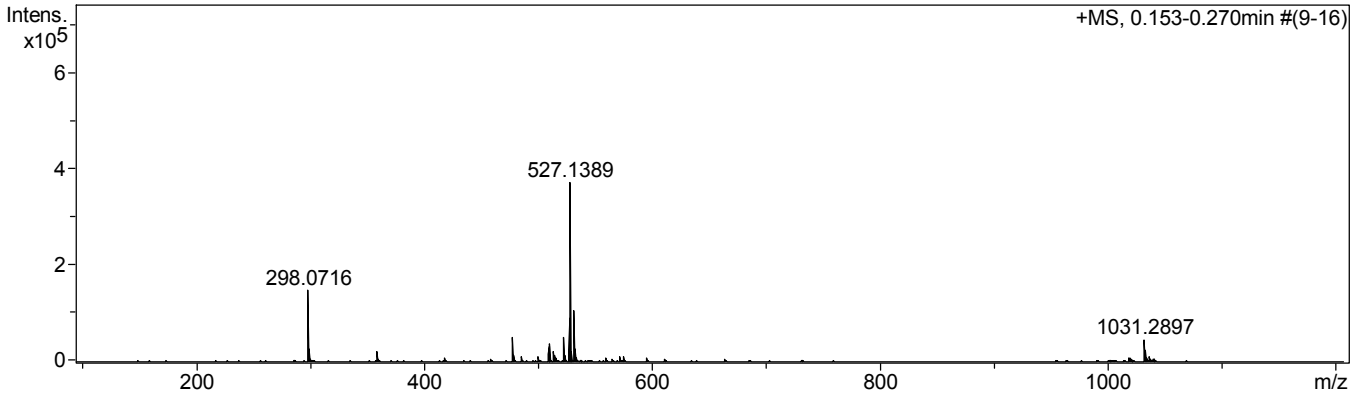
MALDI TOF/TOF HRMS calc'd for $C_{40}H_{59}N_{12}O_{13}S_2^+$ ($M+H^+$): 979.3766, found: 979.4477.

High-Resolution Mass Spectra

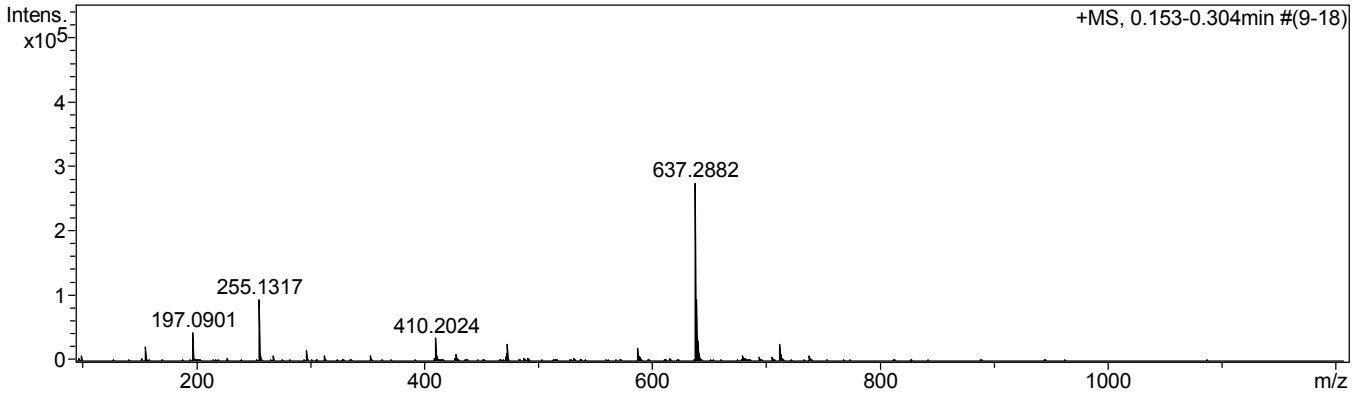
Compound 5



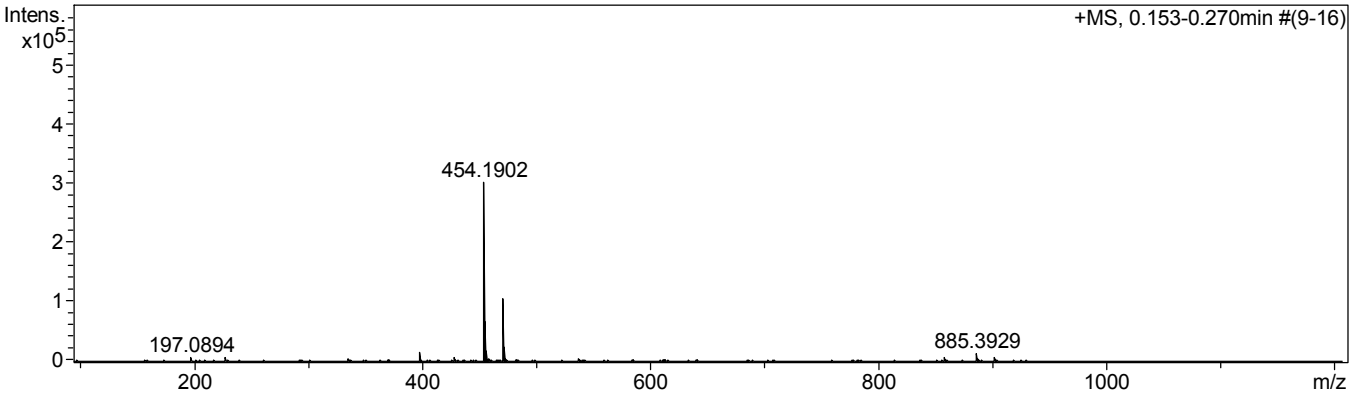
Compound 6



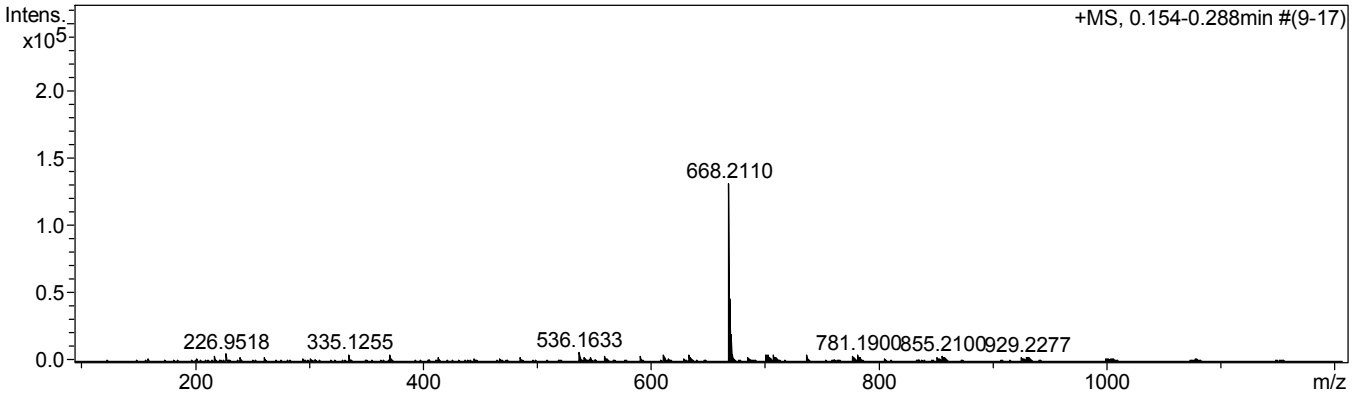
Compound 7



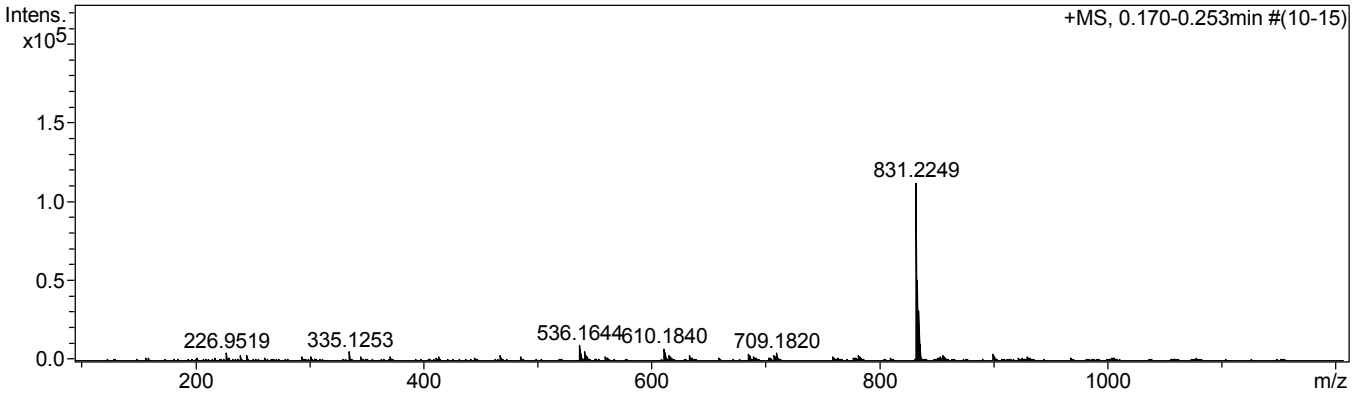
Compound 8



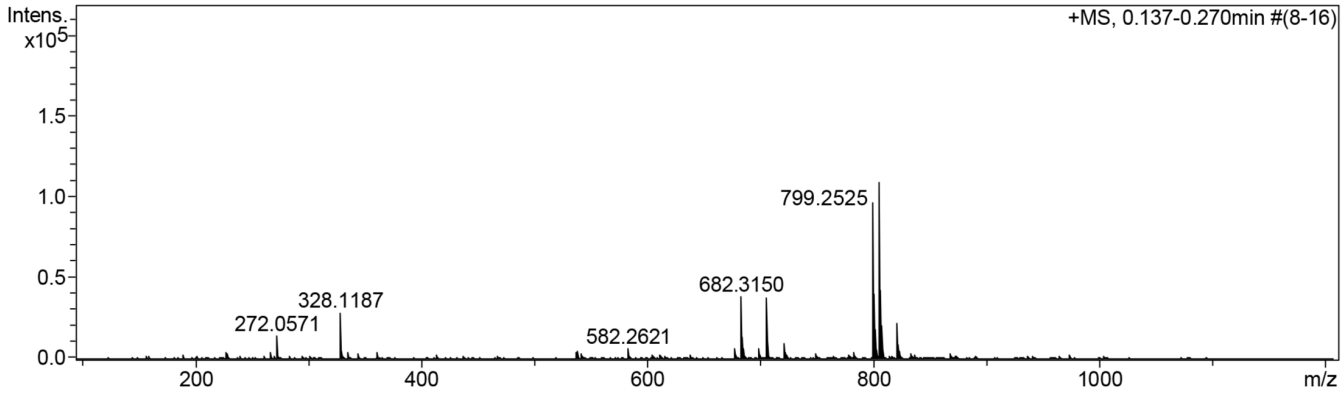
Compound 9



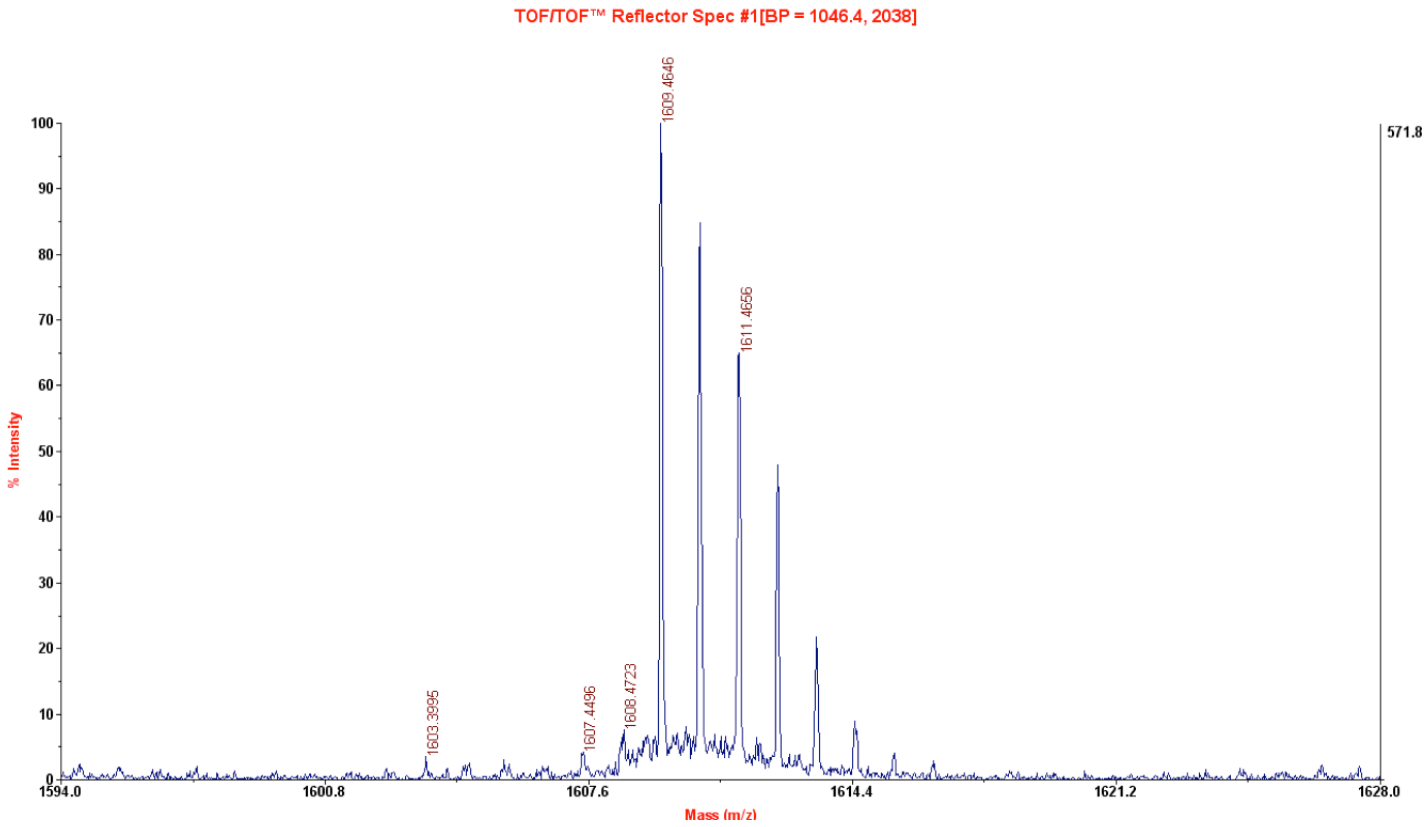
Compound 10



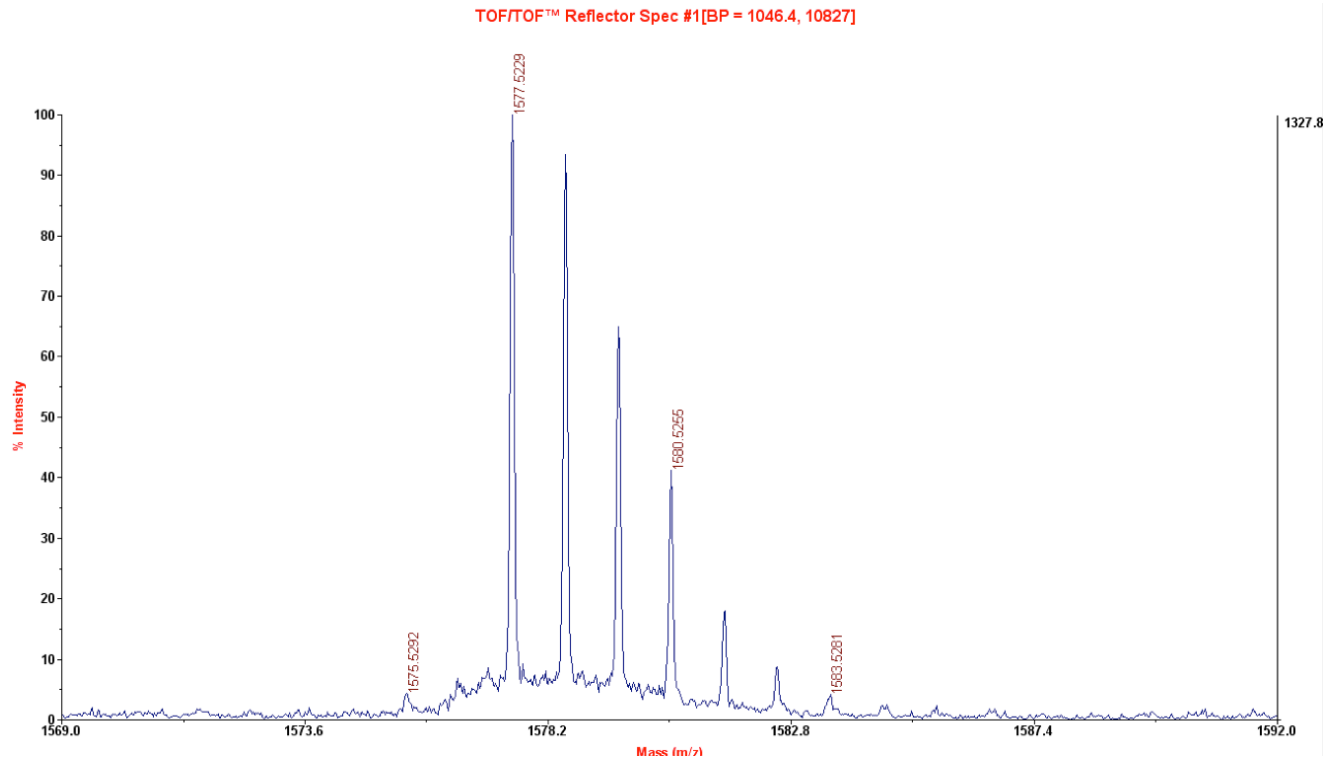
Compound 11



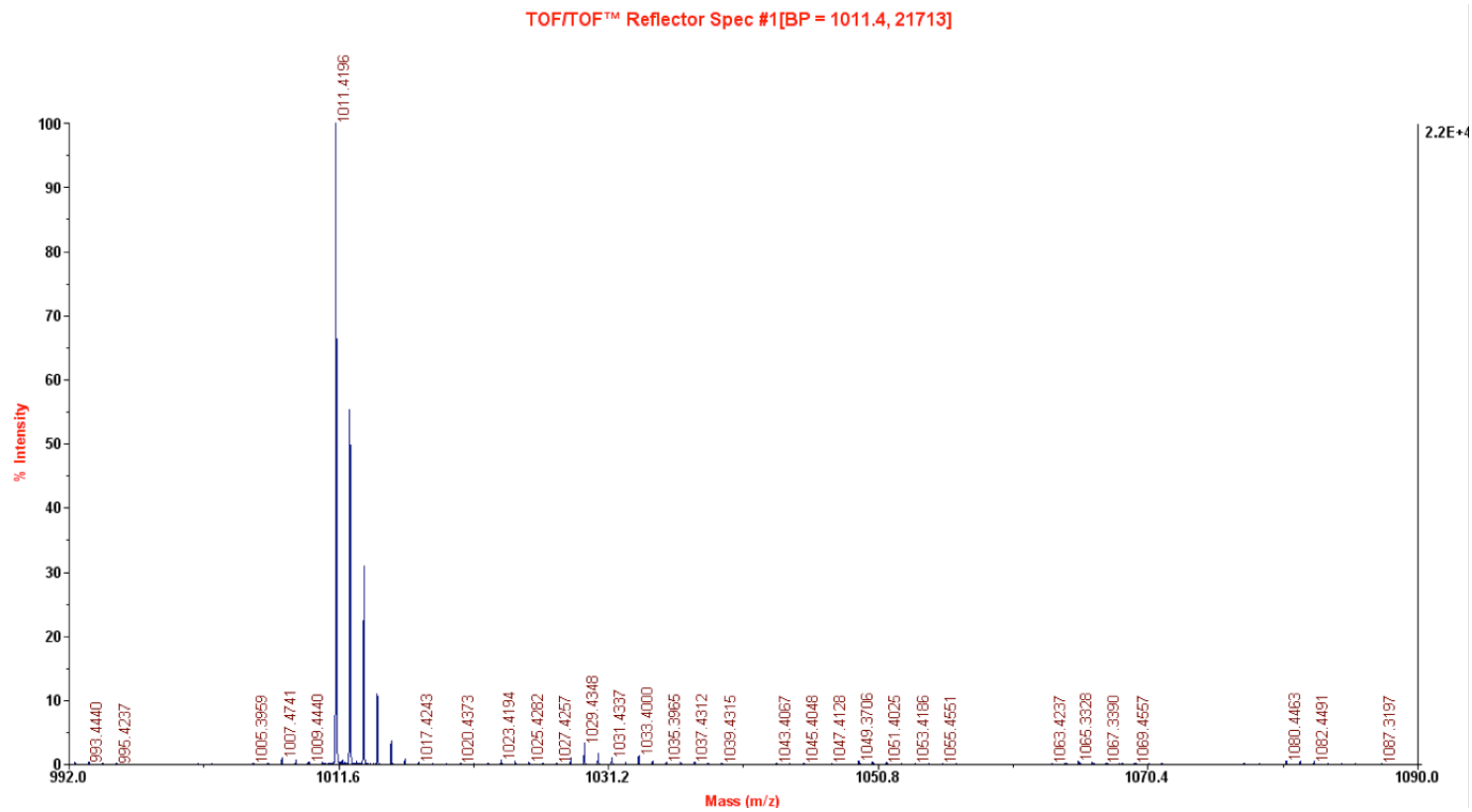
Compound 1



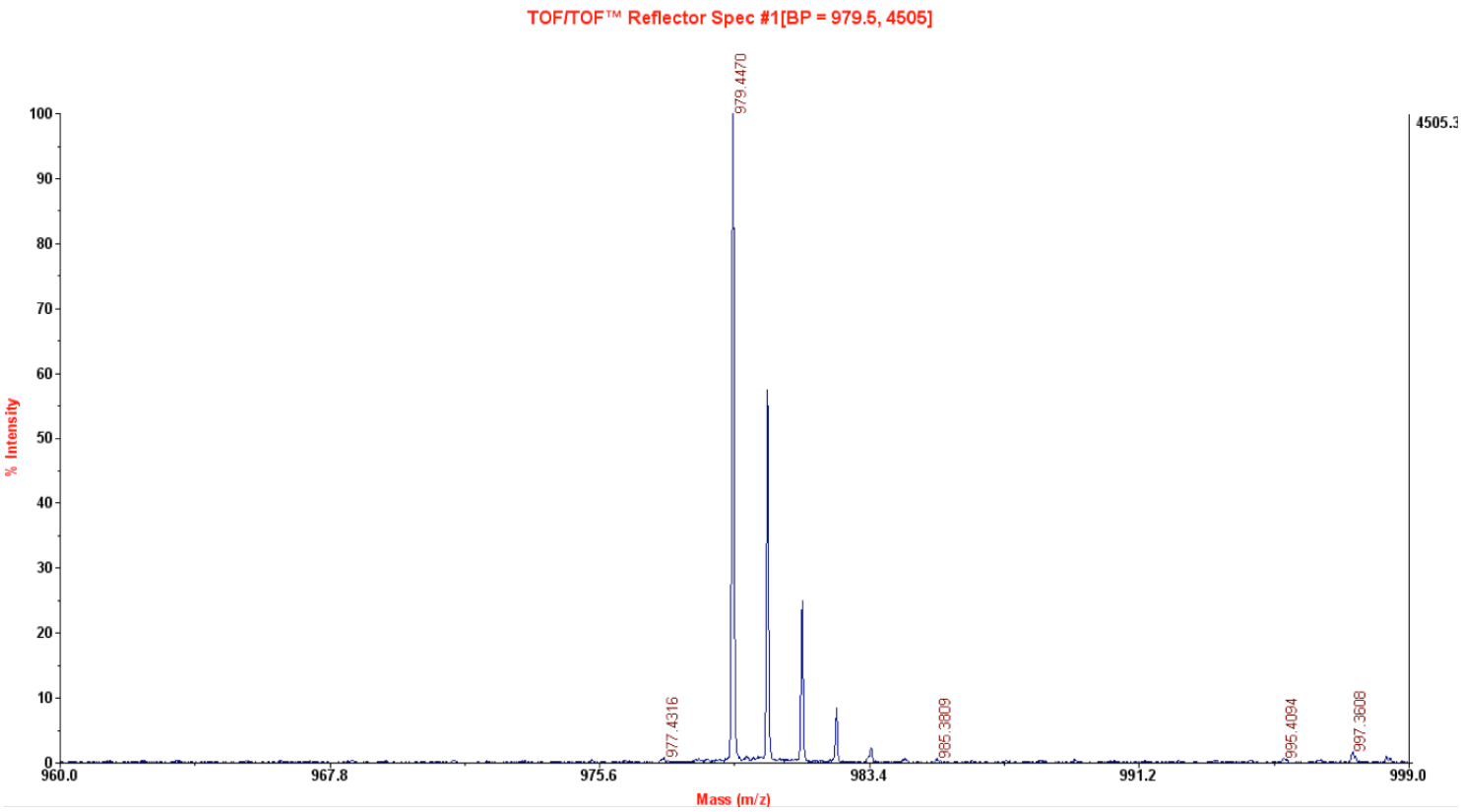
Compound 2



Compound 3



Compound 4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Mass (m/z)
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90
80
70
60
50
40
30
20
10
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975.6
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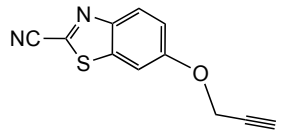
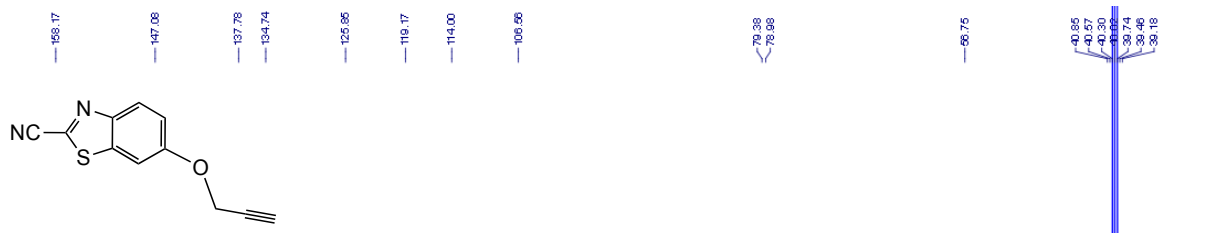
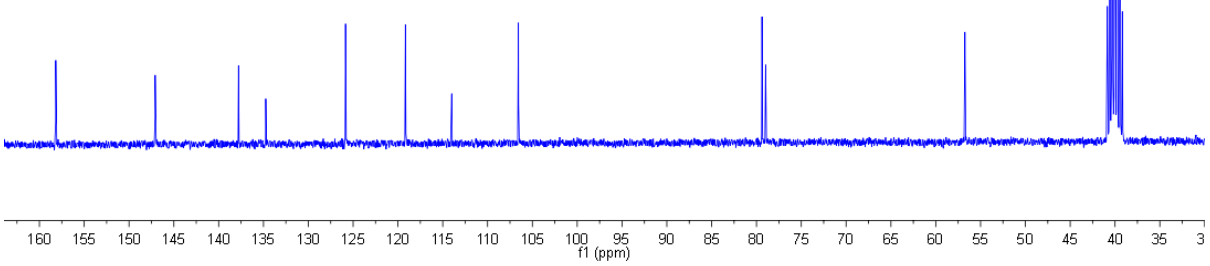
S11.4. NMR Spectra

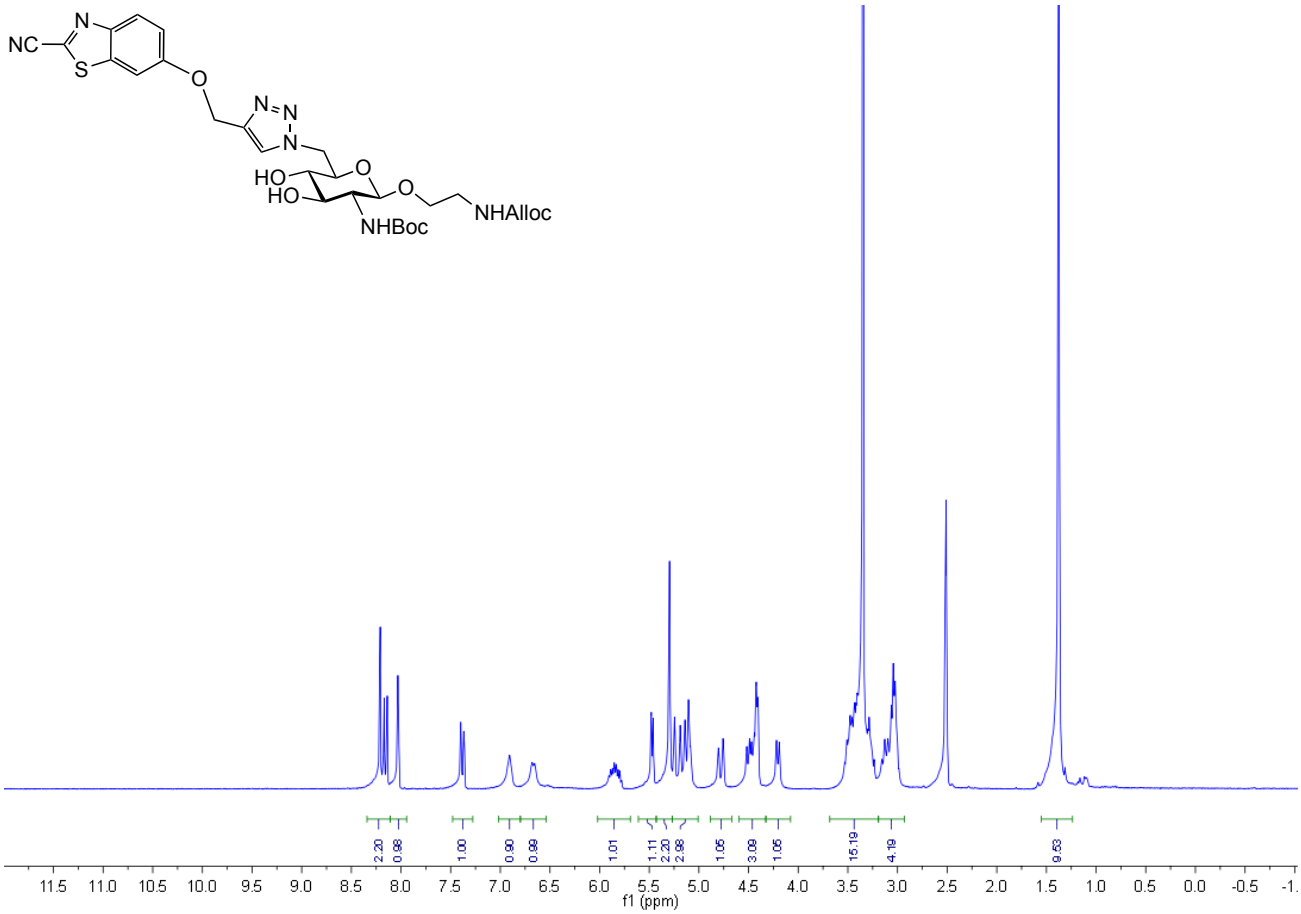
Compound 5 (^{1}H and ^{13}C spectra)

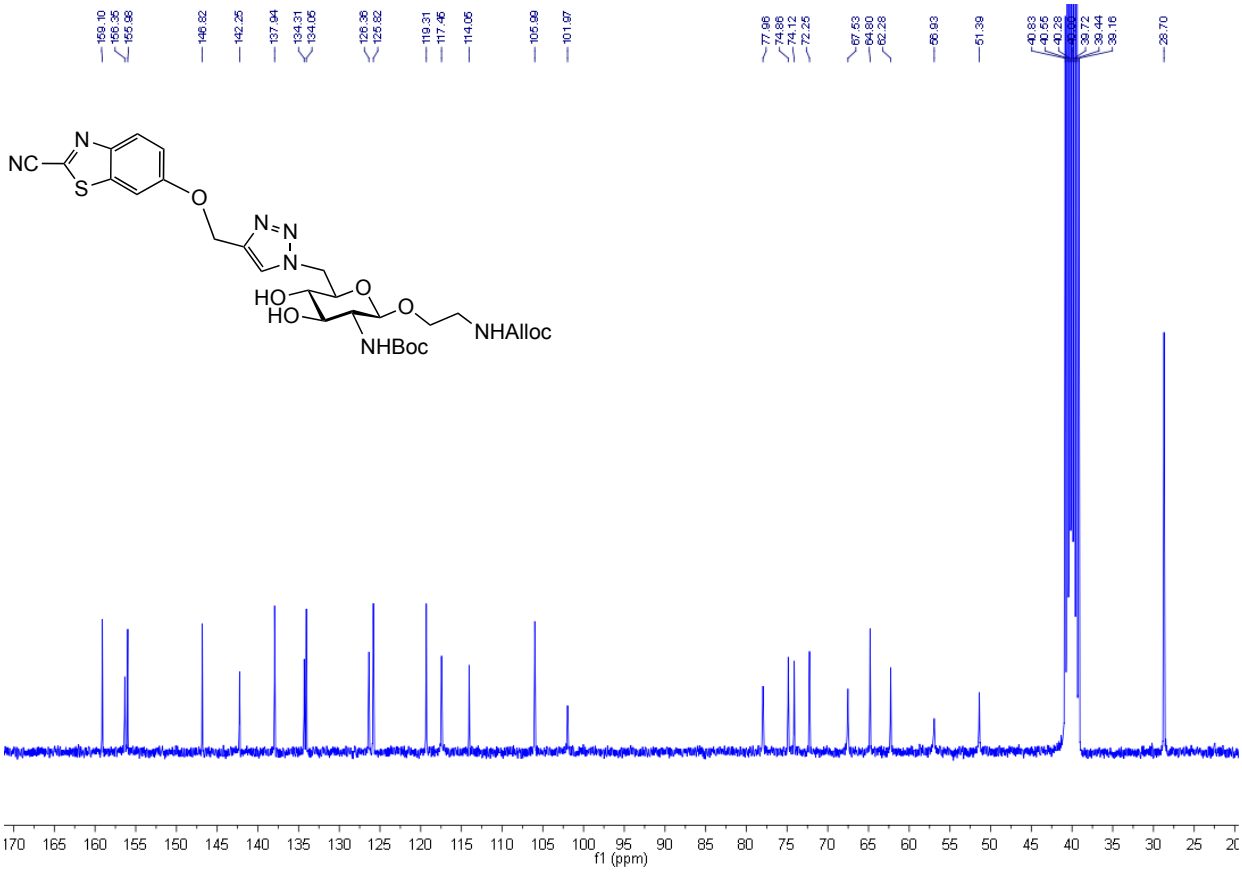


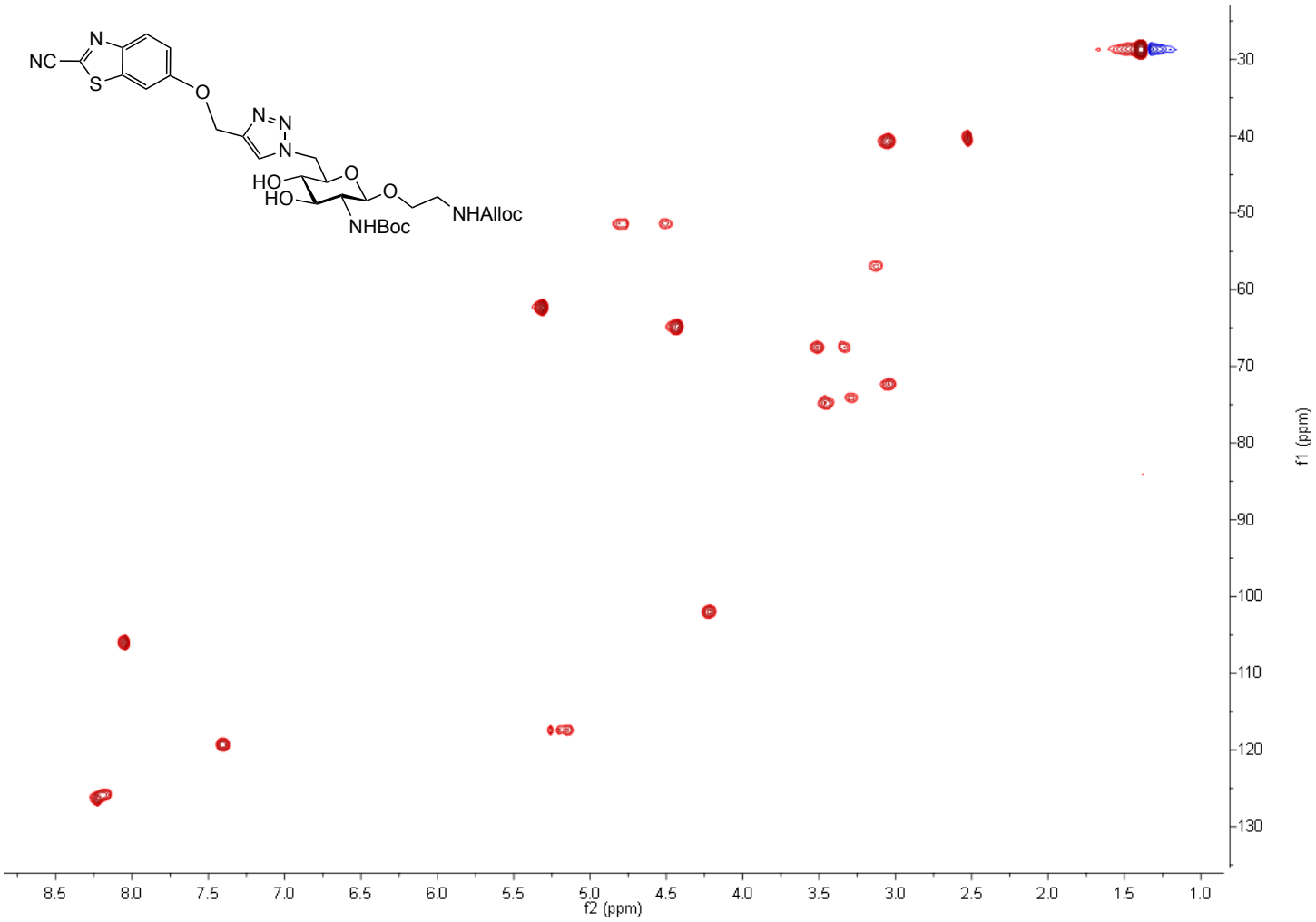




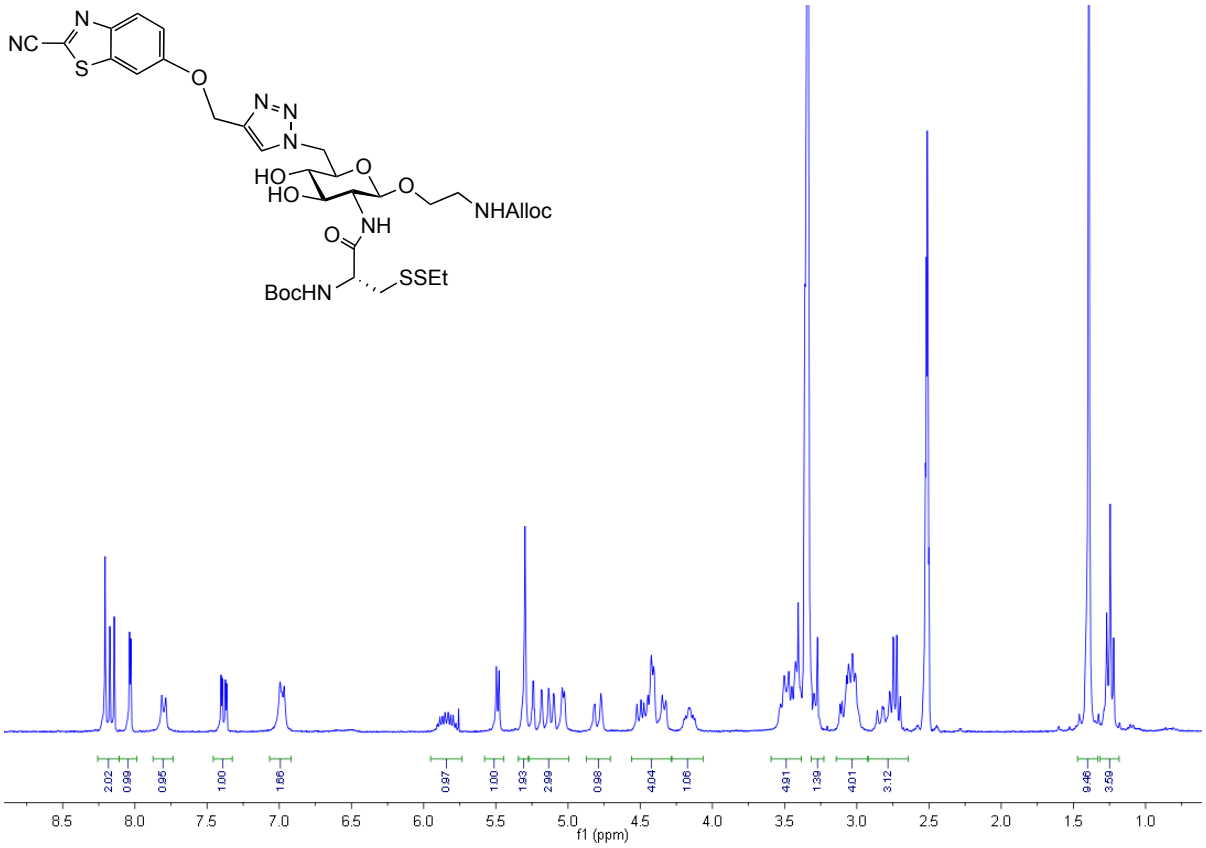
Compound 9 (^{1}H, ^{13}C, and HSQC spectra)

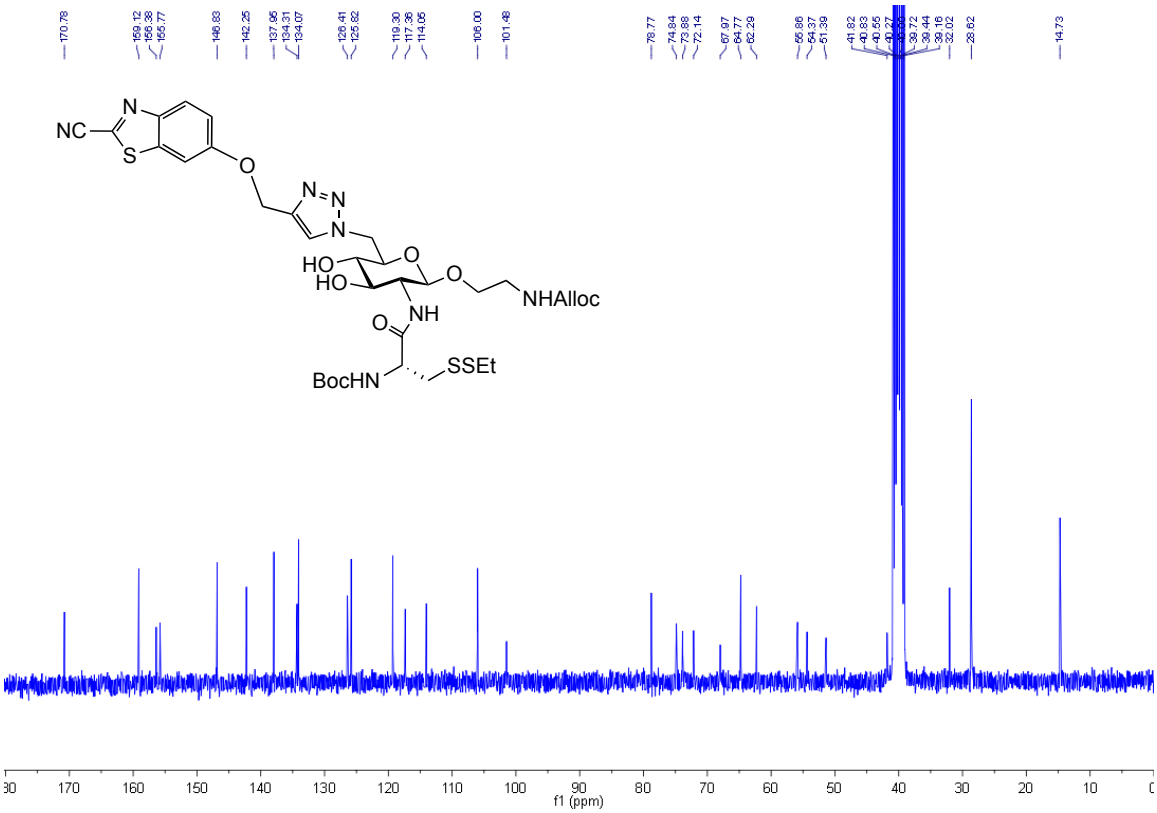


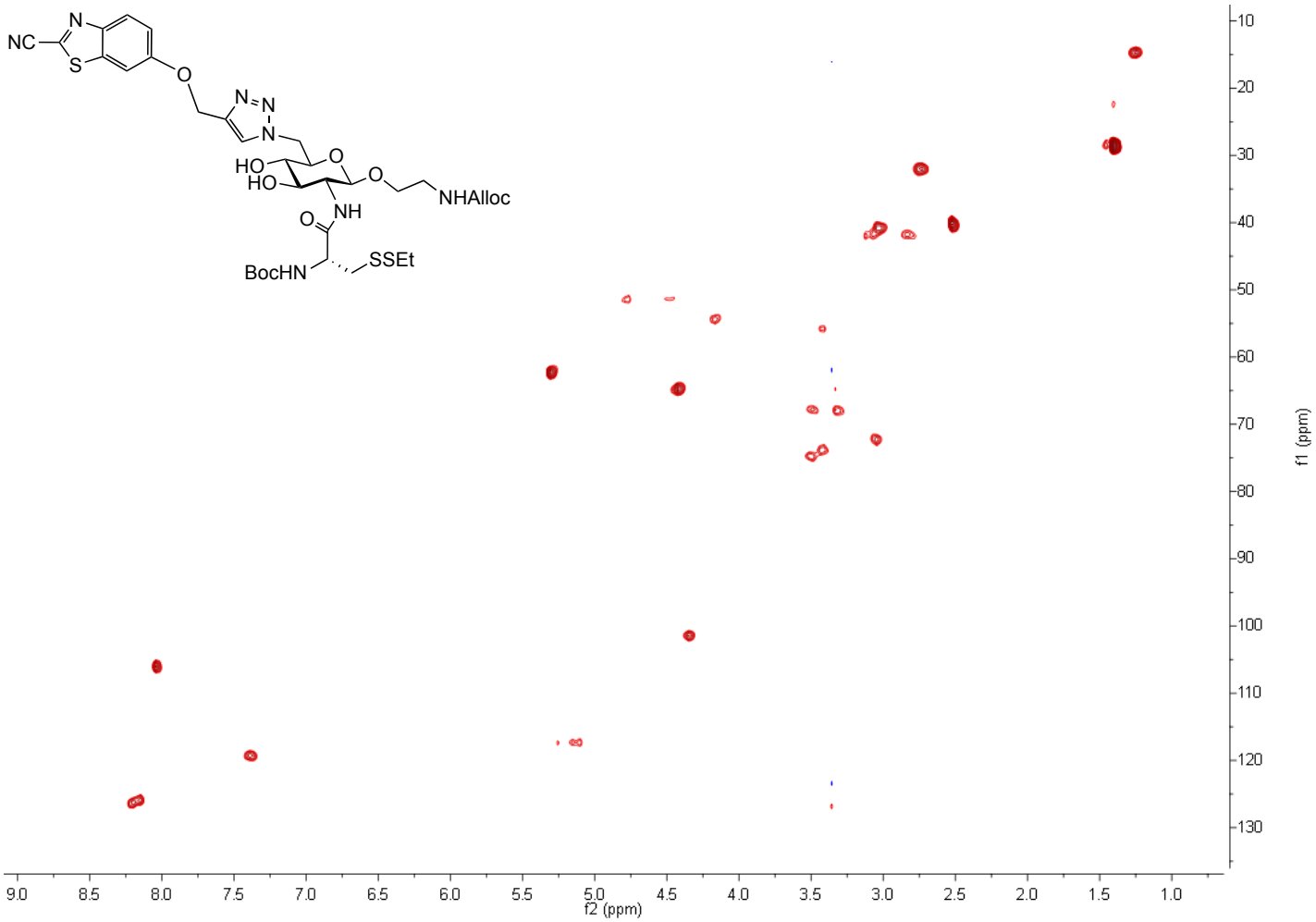



NC
N
S
O
N
N
N
HO
HO
O
O
NHBoc
NHAlloc
f2 (ppm)
f1 (ppm)

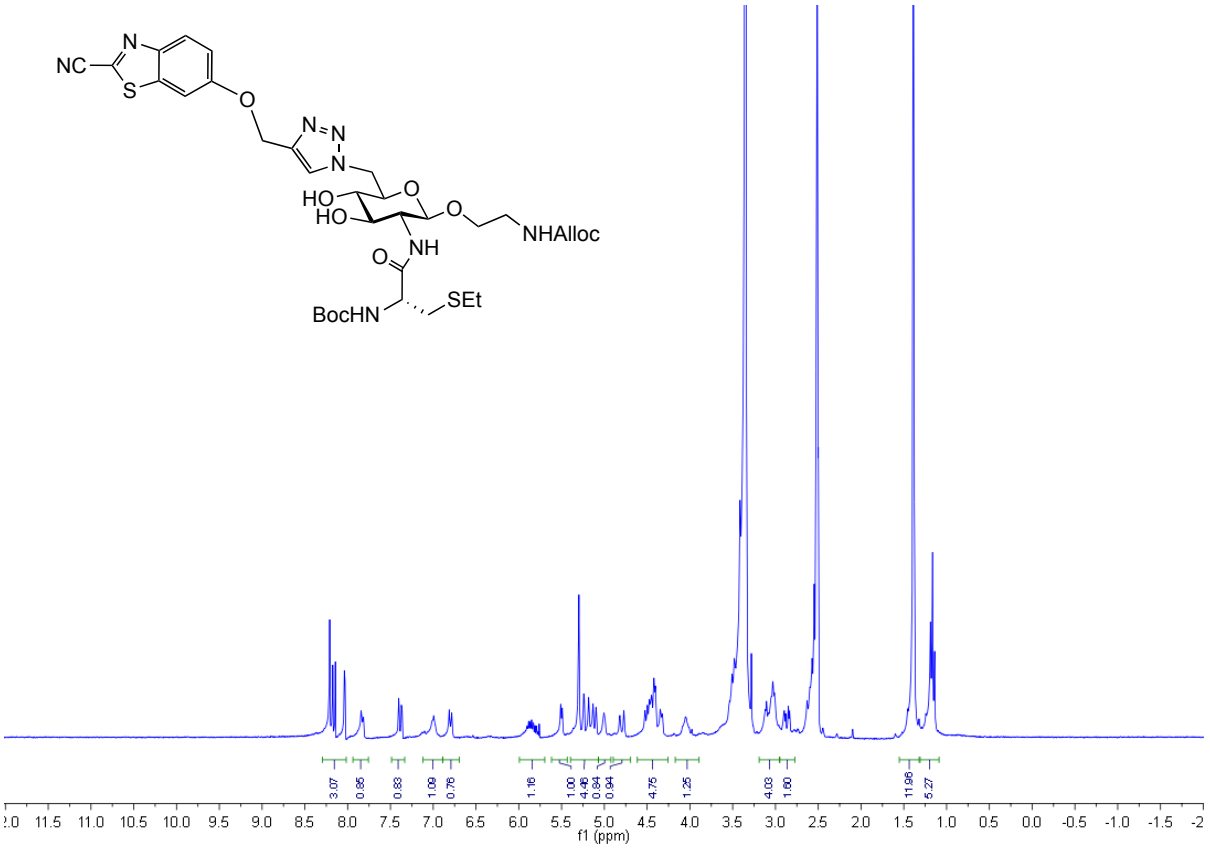
Compound 10 (^{1}H, ^{13}C, and HSQC spectra)

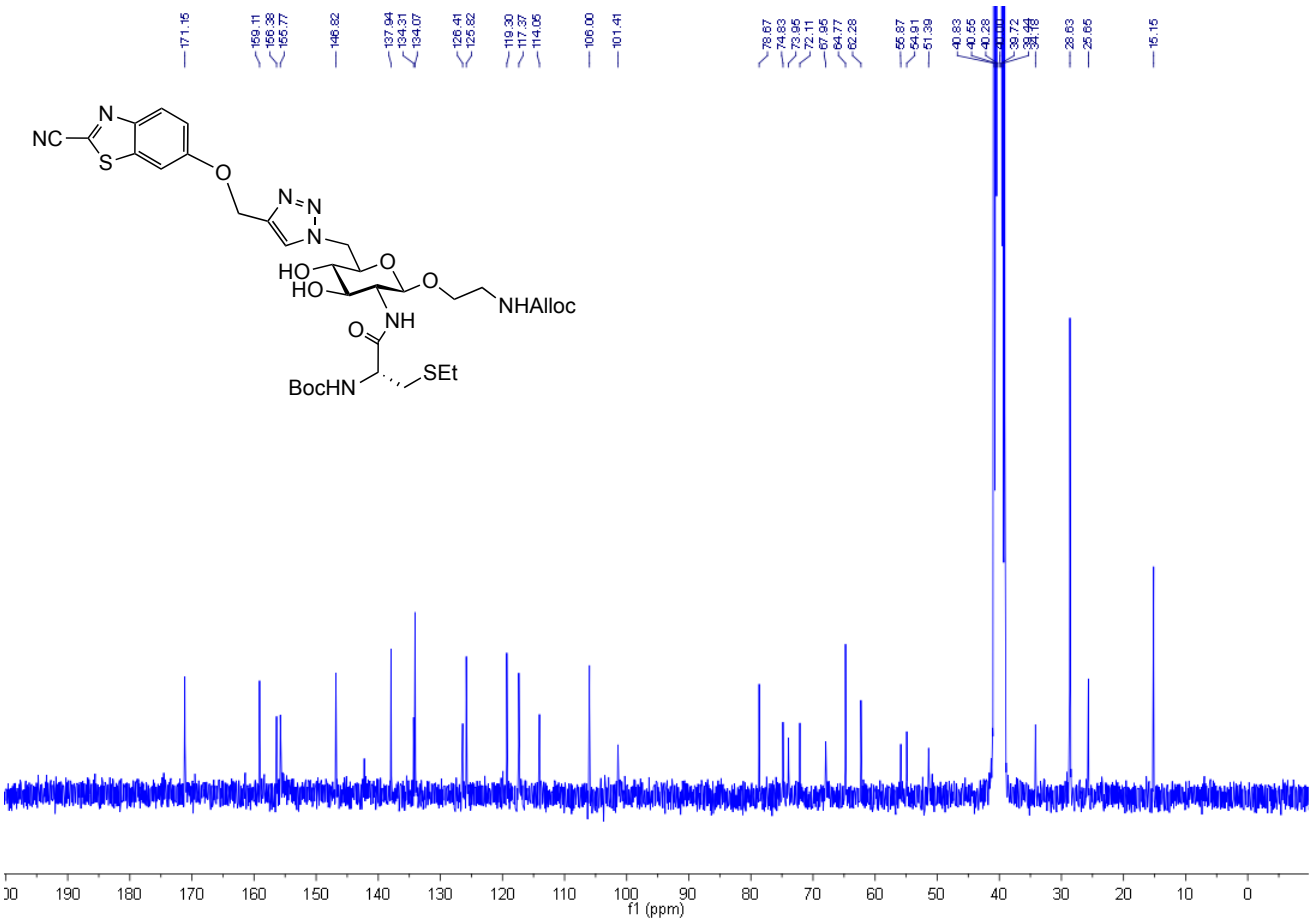


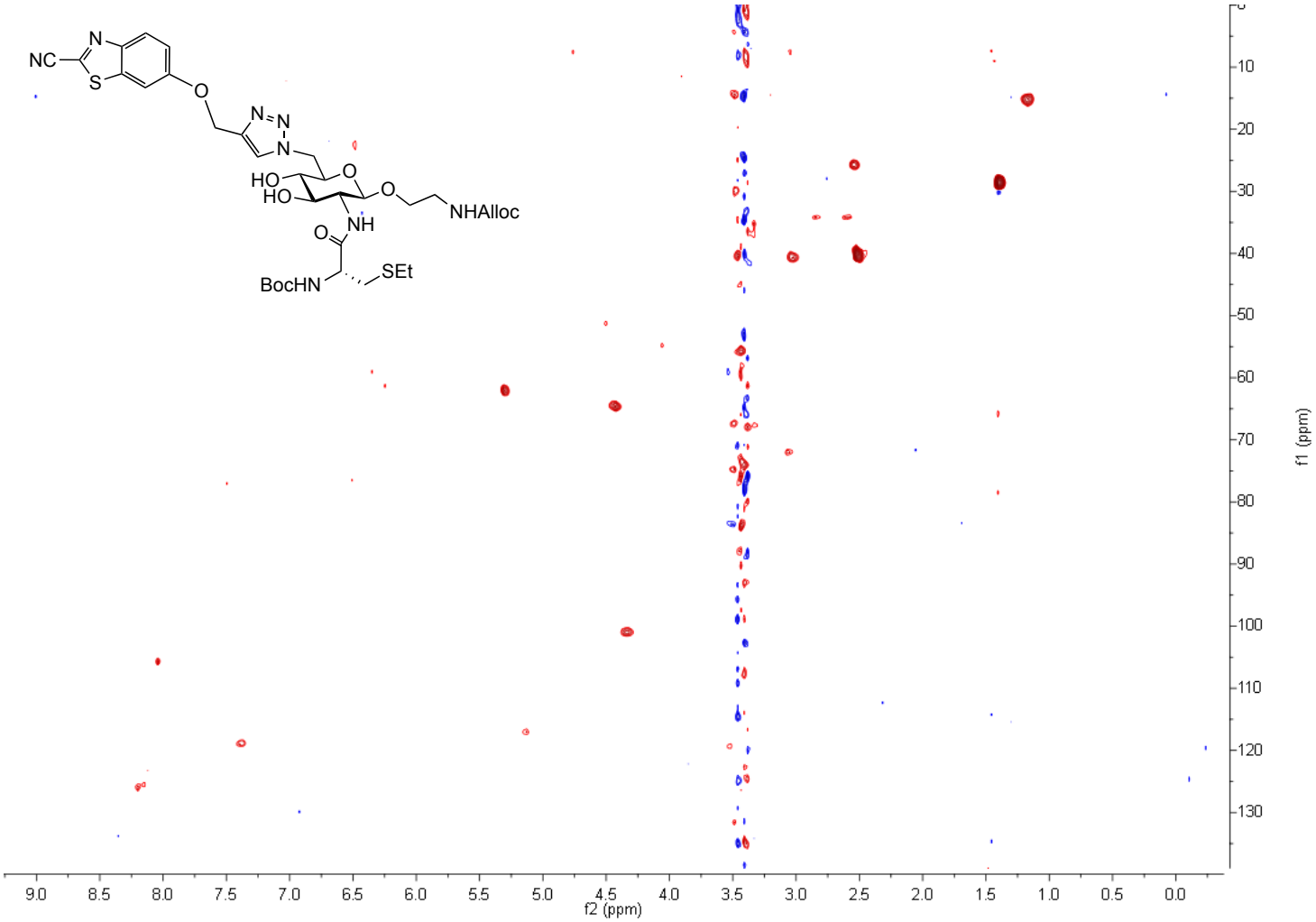



NC
N
S
O
N
N
N
HO
HO
O
O
NHAlloc
O
NH
BocHN
SSEt
f2 (ppm)
f1 (ppm)

Compound 11 (^{1}H, ^{13}C, and HSQC spectra)







References:

(1) Shao, Q.; Jiang, T.; Ren, G.; Cheng, Z.; Xing, B. Photoactivable bioluminescent probes for imaging luciferase activity. Chem. Commun. (Cambridge, U. K.). 2009(27):4028. doi: 10.1039/b908346d.