SUPPLEMENTARY INFORMATION

A Chemoselective Cleavable Fluorescence Turn-ON Linker For Proteomic Studies

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1. General Information

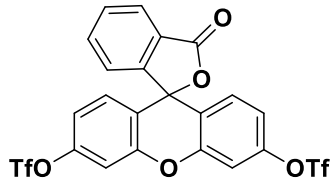
All commercial reagents were purchased from reputable vendors and used without further purification, unless indicated otherwise. Solvents used in palladium cross-couplings were degassed prior to use by sparging with nitrogen for 15 minutes. All reactions were sealed with septa through which a nitrogen atmosphere was introduced unless otherwise stated. Reactions were conducted in round-bottomed flasks or septum capped crimp-top vials containing Teflon-coated magnetic stir bars. Heating of reactions was accomplished with a silicon oil bath on top of a stirring hotplate equipped with an electronic contact thermometer to maintain the indicated temperatures. Reactions were monitored by analytical thin layer chromatography (TLC) on pre-coated silica plates (Merck 60 F254, 0.25 μm) and spots were visualized by UV (254/365 nm) or developed with I_2 or $KMnO_4$ stain. Flash column chromatography was carried out using 200 or 400 mesh silica gel. All analytical HPLC and mass spectra were obtained on Shimadzu LCMS (IT-TOF) or LCMS-2010EV system using reverse-phase Phenomenex Luna 5 μm C18(2) 100 Å 50 × 3.0 mm column. High-resolution mass spectrometry (H-MS) mass spectra (ESI) was obtained on Bruker microTOF-Q II. All NMR spectra were recorded on a 300 or 500 MHz Bruker spectrometer. ^{1}H and ^{13}C chemical shifts (δ) were referenced to TMS or residual solvent peaks ($CDCl_3$ = 7.26 ppm and $(CD_3)_2SO$ = 2.50 ppm) for ^{1}H NMR and ($CDCl_3$ = 77.16 ppm and $(CD_3)_2SO$ = 39.52 ppm) for ^{13}C NMR. The following abbreviations were used for reporting ^{1}H NMR spectra: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constant (Hz), integration.

HeLa and MCF-7 cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich) supplemented with 10% (v/v) fetal bovine serum (FBS; Invitrogen) and 1% (v/v) penicillin-streptomycin (Thermo). All cell lines were grown in a humidified incubator at 37 ºC with 5% CO_2 atmosphere. Protein concentration was determined by Nanodrop™ 2000c spectrophotometer (Thermo). All other reagents used were from available commercial sources, unless otherwise specified. Click regents Tris (2-carboxyethyl) phosphine (TCEP), and Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) were purchased from Sigma-Aldrich. *L*-HPG, Rh-Biotin-Azide and Diazo-Biotin-azide were purchased from Click Chemistry Tools. High-capacity NeutrAvidin™ agarose beads (#29204) was purchased from Thermo Scientific. Trypsin Digestion Kit was purchased from Pierce. In-gel fluorescence scanning of the SDS-PAGE gels was carried out with Typhoon 9410 fluorescence gel scanner (Amersham Biosciences). UV-vis absorption was measured by UV 2550 spectrometer (Shimadzu). Antibody against PDI (sc-166474) was purchased from Santa Cruz Biotechnology Antibodies used for Western blotting (WB) are from the following sources: *anti*-GADPH (Santa Cruz, sc-25778), HRP-conjugated *anti*-rabbit (Thermo, #31460), HRP-conjugated *anti*-mouse (Invitrogen, #626520). The blot was developed using SuperSignal West Dura Extended Duration Substrate (Thermo), and imaged with ImageQuant LAS 500 scanner (GE Healthcare).

2. Chemistry

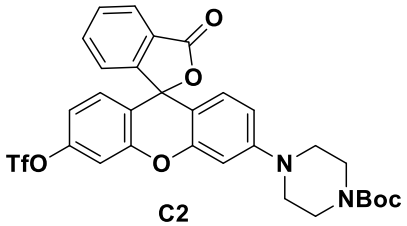
2.1 Synthesis Procedure

Compound 1 **(C1)** was synthesized based on reported procedures.[1]



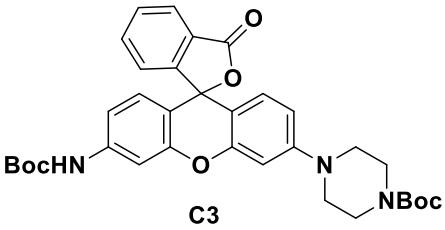
C1

Fluorescein (9.96 g, 30.0 mmol) was suspended in 150 mL dichloromethane. Pyridine (9.6 mL, 120.0 mmol) was slowly added to the resulting suspension at 0 °C. The mixture was stirred at 0 °C for 10 min followed by the dropwise addition of trifluoromethane sulfonic anhydride (12.6 mL, 75 mmol). The reaction mixture was slowly warmed to room temperature and left to stir for 2 h. The reaction mixture was diluted with 200 mL dichloromethane and quenched with 100 mL H_2O. The organic layer was washed with 1 M HCl (100 mL x 5), brine (100 mL x 1) and dried over sodium sulphate. After the solvent was removed in vacuo, the crude residue was recrystallized from ethyl acetate and hexanes to afford the pure product **C1** as off-white solids with 77% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09-8.06 (m, 1H), 7.74-7.67 (m, 2H), 7.30 (d, J = 2.4 Hz, 2H), 7.20-7.17 (m, 1H), 7.04 (dd, J = 8.7, 2.4 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H). LC-MS (IT-TOF) calculated for $[M+H]^+$: 596.97, found 596.85.



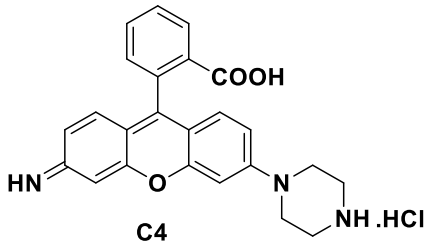
C2

C1 (1.49 g, 2.5 mmol), Boc-piperazine (2.32 g, 12.5 mmol) and Cs_2CO_3 (977 mg, 3.0 mmol) were suspended in 20 mL of anhydrous toluene. The mixture was purged with nitrogen for 10 min followed by the addition of $Pd(dppf)Cl_2.CH_2Cl_2$ (204 mg, 0.25 mmol) and XantPhos (289 mg, 0.50 mmol). The reaction mixture was purged with nitrogen for another 10 min and then stirred at 100 °C for 2-3 h. The reaction was monitored by TLC every 30 min to prevent the formation of side products. The crude reaction mixture was purified by column chromatography using ethyl acetate:hexanes = 0 to 50%. The pure desired product **C2** was obtained as a white solid with 48% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.04 (d, J = 7.2 Hz, 1H), 7.71-7.62 (m, 2H), 7.22 (d, J = 2.4 Hz, 1H), 7.17 (d, J = 7.2 Hz, 1H), 6.95-6.86 (m, 2H), 6.72-6.62 (m, 3H), 3.59-3.56 (m, 4H), 3.24-3.21(m, 4 H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 168.96, 154.61, 152.95, 152.63, 152.25, 151.91, 149.94, 135.22, 130.04, 129.99, 128.69, 126.46, 125.24, 123.84, 120.80, 120.00, 116.45, 112.67, 110.39, 108.73, 102.14, 81.85, 80.11, 48.03, 43.25, 28.38. H-MS (ESI) calculated for $[M+H]^+$: 633.1440, found 633.1511.



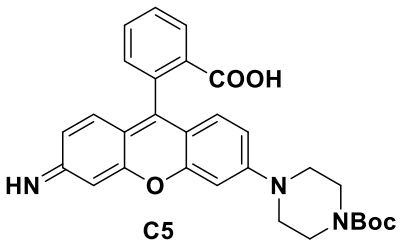
C3

C2 (300 mg, 0.5 mmol), *tert*-butyl carbamate (70 mg, 0.6 mmol) and Cs_2CO_3 (195 mg, 0.6 mmol) were suspended in 8 mL of anhydrous toluene. The mixture was purged with nitrogen for 10 min followed by the addition of $Pd(dppf)Cl_2.CH_2Cl_2$ (40 mg, 0.05 mmol) and XantPhos (58 mg, 0.10 mmol). The reaction mixture was purged with nitrogen for another 10 min and then stirred at 100 °C for 8 h. The crude reaction mixture was purified by column chromatography using ethyl acetate:hexanes = 10 to 50%. The pure desired product **C3** was obtained as a light pink solid with 83% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, *J* = 7.2 Hz, 1H), 7.64-7.59 (m, 2H), 7.52 (s, 1H), 7.13 (d, *J* = 6.9 Hz, 1H), 6.86-6.82 (m, 1H), 6.70-6.58 (m, 5H), 3.58-3.56 (m, 4H), 3.24-3.21 (m, 4 H), 1.52 (s, 9H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 169.52, 154.66, 153.26, 152.72, 152.47, 152.32, 152.13, 140.49, 134.85, 129.56, 128.71, 128.55, 126.81, 124.93, 123.91, 113.97, 113.49, 112.13, 109.60, 106.09, 102.46, 83.34, 81.06, 80.05, 48.25, 43.27, 28.41, 28.27. H-MS (ESI) calculated for $[M+H]^+$: 600.2632, found 600.2700.



C4

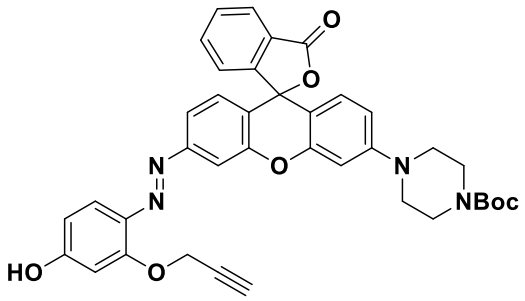
C3 (600 mg, 1.0 mmol) was dissolved in 5 mL dichloromethane followed by the addition of 2.5 mL 4M HCl in 1,4-dioxane. The reaction was left to stir for 6 h at room temperature. Upon completion (monitored by LCMS), the solvent was removed in vacuo and the crude product **C4** was used immediately in the next step as a hydrochloride salt without further purification. LC-MS (IT-TOF) calculated for $[M+H]^+$: 399.16, found 399.95.



C5

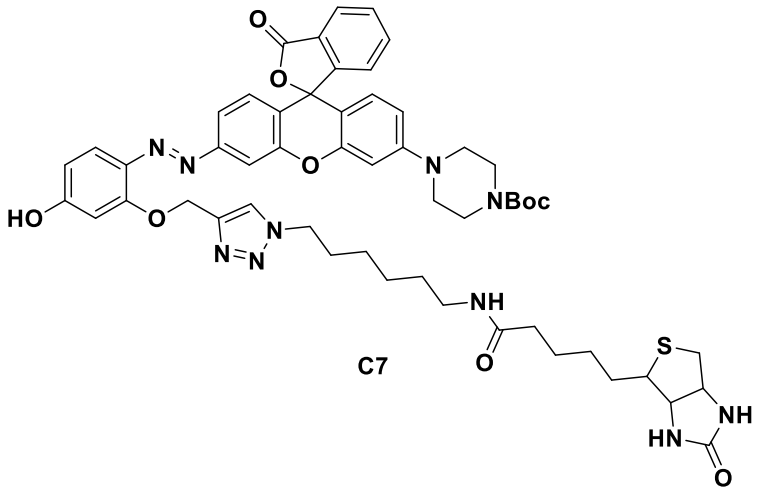
C4 (400 mg, 1.0 mmol) was suspended in 5 mL dichloromethane. Excess triethylamine (1.4 mL, 10.0 mmol) was added and the solids dissolved to give a deep purple solution. Boc anhydride (240 mg, 1.1 mmol) was added and the reaction was stirred at room temperature for 1 h. Upon completion (monitored by TLC), the reaction mixture was diluted with 50 mL dichloromethane, washed with water (50 mL x 2), brine (50 mL x

1) and dried with sodium sulphate. The solvent was removed in vacuo and the crude product was purified with column chromatography using ethyl acetate:hexanes = 50 to 80%. The pure desired product **C5** was obtained as a dark pink solid with 80% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.96 (d, J = 7.5 Hz, 1H), 7.80-7.66 (m, 2H), 7.23 (d, J = 7.8 Hz, 1H), 6.78-6.68 (m, 2H), 6.49 (d, J = 8.7 Hz, 1H), 6.41 (d, J = 1.8 Hz, 1H), 6.36-6.28 (m, 2H), 5.63 (s, 2H), 3.43 (b, 4H), 3.21 (b, 4H), 1.41 (s, 9H). ^{13}C NMR (DMSO-d_6, 300 MHz) δ 168.72, 153.78, 152.42, 152.19, 152.14, 151.99, 151.15, 135.22, 129.74, 128.33, 128.21, 126.51, 124.33, 123.88, 111.57, 110.98, 108.98, 105.57, 101.45, 99.12, 84.06, 78.95, 47.21, 42.80, 27.98. H-MS (ESI) calculated for $[M+H]^+$: 500.2107, found 500.2187.



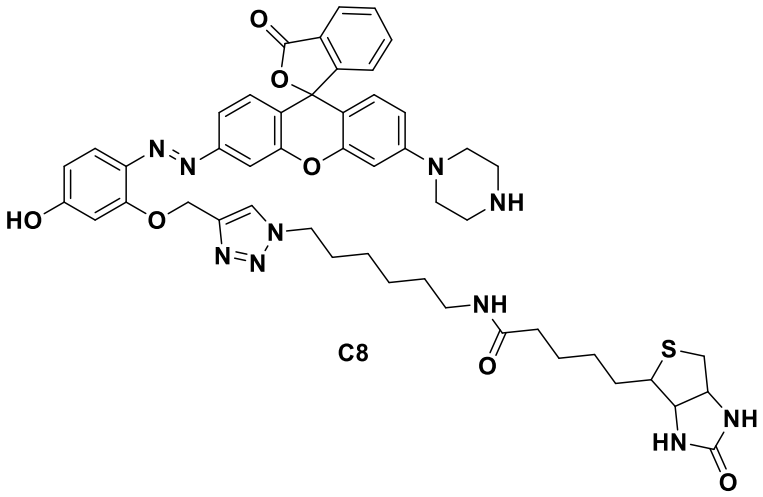
C6

C5 (100 mg, 0.20 mmol) was dissolved in 5 mL dichloromethane:acetonitrile = 1:4 with TFA (0.05 mL, 1% v/v) at 0 ºC. Sodium nitrite (30 mg, 0.24 mmol) was added and the reaction was stirred at 0 ºC for 10 to 15 minutes. In a separate round bottom flask, 3-(prop-2-yn-1-yloxy)phenol (60 mg, 0.4 mmol) was dissolved in 1 mL acetonitrile, followed by the addition of 0.5 mL of sodium carbonate (160 mg dissolved in 5 mL water) and 0.5 mL sodium hydroxide (120 mg dissolved in 5 mL). The 3-(prop-2-yn-1-yloxy)phenol solution was then added in a dropwise manner to diazotized **C5** at 0 ºC. The reaction was stirred for 30 min at 0 ºC. Upon completion, the reaction mixture was diluted with 100 mL of dichloromethane, washed with water (50 mL x 2), brine (50 mL x 1) and dried with sodium sulphate. The solvent was removed in vacuo and the crude product was purified with column chromatography using dichloromethane:methanol = 0 to 1%. The pure desired product **C6** was obtained as a dark purple solid with 58% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.58 (b, 1H), 8.04 (d, J = 7.5 Hz, 1H), 7.84-7.72 (m, 2H), 7.64-7.61 (m, 2H), 7.49 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 7.2 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 6.89 (s, 1 H), 6.79 (d, J = 9.0 Hz, 1H), 6.69 (s, 1H), 6.61 (d, J = 9.0 Hz, 1H), 6.52 (d, J = 7.2 Hz, 1H), 4.98 (s, 2H), 3.66 (s, 1H), 3.45 (b, 4H), 3.24 (b, 4H), 1.42 (s, 9H). ^{13}C NMR (DMSO-d_6, 300 MHz) δ 168.57, 163.24, 157.52, 153.96, 153.81, 152.50, 152.39, 151.68, 151.34, 135.70, 135.27, 130.25, 128.88, 128.36, 125.71, 124.78, 123.94, 120.01, 117.95, 117.79, 112.21, 109.49, 109.25, 107.86, 101.95, 101.33, 82.14, 79.01,78.96, 78.71, 56.68, 47.08, 42.80, 28.00. H-MS (ESI) calculated for $[M+H]^+$: 659.2506, found 659.2427.



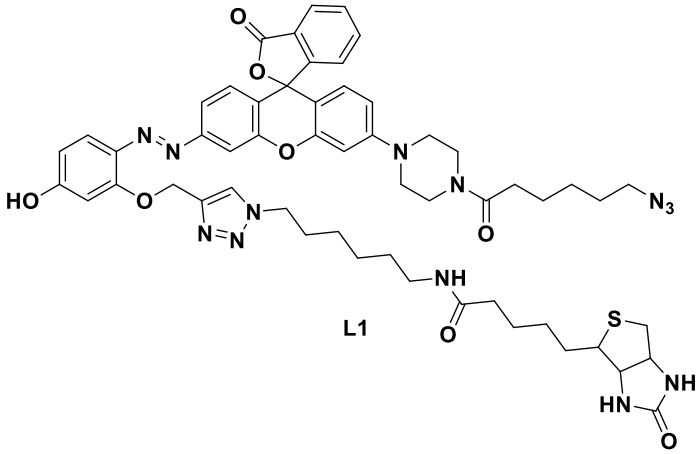
C7

C6 (33 mg, 0.05 mmol) and N-(6-azidohexyl)-5-(2-oxohexahydro-1H-thieno[3,4-d] imidazol-4-yl)pentanamide (18 mg, 0.05 mmol) were dissolved in 1.5 mL *t*-butanol. Tris(3-hydroxypropyltriazolylmethyl)amine (2 mg, 0.005 mmol) and copper (II) sulphate (12 mg, 0.075 mmol) were dissolved in 0.4 mL water and added to the reaction mixture. Sodium ascorbate (15 mg, 0.075 mmol) was also dissolved in 0.4 mL water and added dropwise (to prevent precipitation) to the reaction mixture. The reaction was stirred at room temperature for 10 min. Upon completion (monitored by TLC), the reaction mixture was diluted with 50 mL of dichloromethane, washed with water (20 mL x 3), brine (20 mL x 1) and dried with sodium sulphate. The solvent was removed in vacuo and the crude product was purified with column chromatography using dichloromethane:methanol = 0 to 5%. The pure desired product **C7** was obtained as a dark purple solid with 68% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.53 (b, 1H), 8.22 (s, 1H), 8.04 (d, J = 7.4 Hz, 1 H), 7.83-7.66 (m, 3H), 7.62-7.59 (m, 2H), 7.45 (dd, J = 8.4 Hz, 1.7 Hz, 1H), 7.31 (d, J = 7.26 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.88-6.87 (m, 1H), 6.79-6.77 (m, 2H), 6.61 (d, J = 8.8 Hz, 1H), 6.49 (dd, J =8.9 Hz, 2.0 Hz, 1H), 6.40 (s, 1H), 6.34 (s, 1H), 5.33 (s, 2H), 4.37-4.26 (m, 3 H), 4.12-4.08 (s, 1H), 3.45 (b, 4H), 3.25 (b, 4H), 3.09-3.03 (m, 1H), 2.99-2.93 (q, 2H), 2.82-2.76 (m, 1H), 2.57-2.53 (m, 1H), 2.02 (t, 2H), 1.80-1.76 (m, 2H), 1.63-1.45 (m, 4H), 1.42 (s, 9H), 1.32-1.22 (m, 8H). ^{13}C NMR (DMSO-d_6, 300 MHz) δ 171.74, 168.57, 163.42, 162.62, 158.44, 153.99, 153.81, 152.50, 152.38, 151.66, 151.31, 142.46, 135.71, 135.35, 130.25, 128.83, 128.37, 125.71, 124.76, 124.35, 123.94, 119.85, 117.83, 117.59, 112.23, 109.77, 109.02, 107.83, 102.10, 101.30, 82.15, 79.01, 62.69, 61.00, 59.16, 55.34, 49.29, 47.08, 38.17, 35.16, 29.55, 28.85, 28.13, 28.01, 25.67, 25.46, 25.25. H-MS (ESI) calculated for $[M+Na]^{+}$: 1049.4348, found 1049.4422.



C8

C7 (110 mg, 0.10 mmol) was suspended in 1 mL dichloromethane and 0.2 mL of trifluoracetic acid (20% v/v) was added. The reaction was stirred at room temperature and monitored by LCMS. Upon completion, the reaction mixture was diluted with 100 mL dichloromethane, washed with saturated sodium bicarbonate (50 mL x 3), brine (50 mL x 1) and dried with sodium sulphate. The solvent was removed in vacuo and the crude product **C8** was used immediately in the next step without further purification. LC-MS (IT-TOF) calculated for $[M+H]^+$: 928.09, found 928.35.



L1

C8 (crude, 0.10 mmol) and 6-azidohexanoic acid (23.6 mg, 0.15 mmol) was dissolved in 2 mL dimethylformamide. Triethylamine (70 uL, 0.50 mmol) was added followed by N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (28.8 mg, 0.15 mmol). The reaction was stirred at room temperature for 4 h. Upon completion, the reaction mixture was diluted with 50 mL dichloromethane and washed with 1M HCl (20 mL x 3), brine (20 mL x 1) and dried with sodium sulphate. The solvent was removed in vacuo and the crude product was purified using column chromatography using dichloromethane:methanol = 0 to 7%. The pure desired product **L1** was obtained as a dark orange solid with 62% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.54 (b, 1H), 8.22 (s, 1H), 8.04 (d, J = 7.3 Hz, 1 H), 7.83-7.67 (m, 3H), 7.62-7.59 (m, 2H), 7.45 (d, J = 9.3 Hz, 1H), 7.31 (d, J = 7.3 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.86 (s, 1H), 6.80-6.77 (m, 2H), 6.62 (d, J = 8.8 Hz, 1H), 6.49 (dd, J = 8.9 Hz, 1.2 Hz, 1H), 6.42 (s, 1H), 6.35 (s, 1H), 5.33 (s, 2H), 4.36-4.26 (m, 3 H), 4.12-4.08 (s, 1H), 3.58 (b, 4H), 3.31-3.23 (b, 5H), 3.09-3.03 (m, 1H), 2.99-2.93 (q, 2H), 2.82-2.76 (m, 1H), 2.57-2.54 (m, 1H),

2.35 (t, 2H), 2.02 (t, 2H), 1.80-1.76 (m, 2H), 1.54-1.41 (m, 8H), 1.38-1.27 (m, 11H). ^{13}C NMR (DMSO-d_6, 300 MHz) δ 171.73, 170.56, 168.55, 163.34, 162.61, 158.42, 153.98, 152.38, 151.67, 151.31, 142.45, 135.69, 135.38, 130.23, 128.82, 128.38, 125.71, 124.76, 124.33, 123.93, 119.88, 117.82, 117.60, 112.08, 109.77, 108.97, 107.78, 102.09, 101.15, 82.15, 62.69, 61.00, 59.15, 55.34, 54.79, 50.56, 49.28, 47.41, 47.05, 44.32, 38.17, 35.15, 31.98, 29.55, 28.85, 28.12, 28.06, 27.97, 25.87, 25.66, 25.46, 25.24, 24.17. H-MS (ESI) calculated for $[M+Na]^+$: 1088.4643, found 1088.4536.

2.2 Structures of Commercially Available Diazo-Biotin-Az and Rh-Biotin-Az Linkers

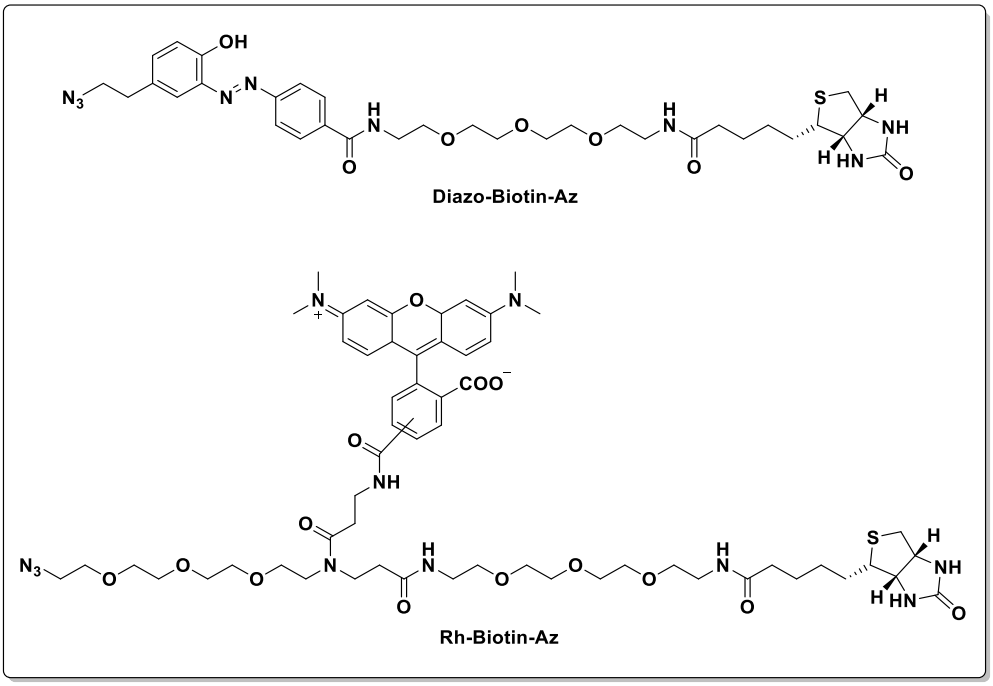


Figure S1. Structures of commercially available linkers, Diazo-Biotin-Azide and Rh-Biotin-Azide, used in our study.

3. Proteomics

3.1 Methods

*In Situ and In Vitro Proteome Labeling with **P1.*** MCF-7 cells were grown to > 90% confluency in 10 cm dishes. After washing with PBS, the cells were treated with 5 μM of **P1** in 5 mL DMEM growth medium with a final DMSO concentration of < 0.1% for 4 h. After incubation, the medium was removed and cells were washed with PBS thrice to remove excess probe. Cells were detached and lysed using 1 mL lysis buffer (0.1% NP40, 0.1 mM PMSF) with sonication carried out on an ice bath. The protein concentration was normalized to 2.5 mg/mL by Nanodrop. 50 μM of linker (from 1 mM DMSO stock) was first added to the lysate, followed by the addition of a freshly premixed click cocktail consisting of 120 μM of THPTA (10 mM in DMSO), 1 mM $CuSO_4$ (100 mM in H_2O) and 1 mM TCEP (100 mM in H_2O).[2] The reaction was incubated at room temperature for 2 h with gentle shaking before termination by addition of five-fold volume pre-chilled acetone (kept overnight at -20 °C). Precipitated proteins were subsequently collected by centrifugation (13, 000 rpm x 15 min at 4 °C),

air dried until it started to shrink, and a pellet was obtained.

*Cleavage Optimisation Using **P1** Labeled Lysate*. After acetone precipitation, the pellet was re-dissolved in 1% SDS and treated with 0, 10, 25, 50, 100 mM of freshly prepared $Na_2S_2O_4$ (dissolved in PBS buffer) for 15 min.[3] After which, 2 × SDS loading buffer without dithiothreitol (DTT) was added and the lysate was boiled for 15 min at 95 °C. The labelled lysate was resolved on 12% SDS-PAGE gel followed by in-gel fluorescence scanning.

Note: We found that the use of DTT under heating conditions resulted in the reduction of azo-rhodamine and hence may confound the $Na_2S_2O_4$ cleavage optimisation process.

In Situ and In Vitro Proteome Labeling with L-HPG. HeLa cells were grown to ~70% confluency in 10 cm dishes. After washing with PBS, the cells were starved with methionine-free DMEM growth medium (Thermo Scientific) for 1 h. The cells were then washed with PBS twice and treated with 100 μM of *L*-Homopropargylglycine (*L*-HPG) in methionine-free DMEM growth medium with a final DMSO concentration of < 0.1% for 8 h. After incubation, the medium was removed and cells were washed with PBS thrice to remove excess probe. Cells were detached and lysed using 1 mL lysis buffer (0.1% NP40, 0.1 mM PMSF) with sonication carried out on an ice bath. The protein concentration was normalized to 2.5 mg/mL by Nanodrop. 50 μM of linker (from 1 mM DMSO stock) was first added, followed by the addition of a freshly premixed click cocktail consisting of 200 μM of THPTA (10 mM in DMSO), 1 mM $CuSO_4$ (100 mM in H_2O) and 1 mM TCEP (100 mM in H_2O).[3] The reaction was incubated at room temperature for 2 h with gentle shaking before termination by addition of five-fold volume pre-chilled acetone (kept overnight at -20 °C). Precipitated proteins were subsequently collected by centrifugation (13, 000 rpm x 15 min at 4 °C). The supernatant was discarded. The pellet was collected, resuspended in pre-chilled methanol by sonication and kept at -20 °C for 1 h. Precipitated proteins were again collected by centrifugation (13, 000 rpm x 15 min at 4 °C). The methanol wash step was repeated twice. The protein was then air-dried until the pellet started to shrink. The pellet was then used for subsequent pull-down and Western Blot experiments as described below.

In Situ Pull-down (PD) for Western Blot (WB) and Quantitative Mass Spectrometry. Air-dried pellets obtained from proteome labelling were solubilized in 6M urea and 1% SDS with sonication before addition to pre-washed NeutrAvidin™ agarose beads (80 μL), followed by incubation at room temperature for 3 h on a rotator. The beads were washed repeatedly in the following sequence with 6 M urea, 1% SDS, 3% SDS, 3% SDS and 1% SDS. For the samples pull-downed with **L1** and Diazo-Biotin-Azide, 25 mM of freshly prepared $Na_2S_2O_4$ (dissolved in PBS buffer with 1% SDS) was then added to the beads for cleavage (3 x 15 min). The tubes were placed on a shaker and the reaction mixture was shaken gently at room temperature. After 15 mins, the avidin beads were centrifuged and the eluate collected. The eluates were either collected/analysed separately in their respective fractions or combined together. 4 x standard SDS-loading buffer was added to the eluates and heated for 10 min at 95°C with gentle mixing. After the third chemical cleavage with $Na_2S_2O_4$, 2 x standard SDS-loading buffer was added to the avidin beads and boiled for 30 min at 95°C with gentle

mixing. Finally, the eluates, combined eluates and boiled beads samples were resolved on a 12% SDS-PAGE gel, followed by in-gel fluorescence scanning. As for the samples pull-downed using Rh-Biotin-Azide, 2 x standard SDS-loading buffer was added to the avidin beads and boiled for 30 min at 95°C with gentle mixing. Finally, all the samples were resolved on 12% SDS-PAGE gel, followed by in-gel fluorescence scanning.

Note: When 25 mM $Na_2S_2O_4$ in PBS (*p*H 7.4) was used, the protein which had been chemically cleaved from the NeutrAvidin™ agarose beads could not dissolve in PBS buffer. We observed that the beads turned pink (rhodamine's turned-on fluorescence upon cleavage) but no fluorescence was detected in the eluate. After washing the beads with 1% SDS, the protein dissolved (detected by in-gel fluorescence scanning).

Western Blotting (WB). For Western blotting (WB) analysis, the PD samples were first resolved on 12% SDS-PAGE gel and transferred to a 0.2 μm PVDF membrane (Thermo). The membrane was blocked with 3% BSA in PBST (0.1% Tween-20) for 1 h at room temperature and washed with TBST (1 x 10 min). After which, the membrane was then incubated with the primary antibody in 3% BSA (PDI 1:500, Santa Cruz) for 1 h at room temperature followed by washing with PBST (3 x 15 min). Then HP-conjugated anti-mouse (Pierce, 1:2000) was applied for 1 h at room temperature followed by washing with PBST (3 x 15 min). The blot was developed using SuperSignal West Dura Extended Duration Substrate (Thermo Fischer), and finally imaged with an ImageQuant LAS 500 scanner (GE Healthcare).

Quantitative Mass Spectrometry. Pull-down (PD) experiments were similarly prepared as described in the above procedures using the respective linkers. The PD samples were then separated by SDS-PAGE gel and subject to Coomassie Brilliant Blue (CBB) staining. The strongly fluorescently-labeled/CBB-stained bands were excised from the gel. The proteins were subjected to tryptic in-gel digest digestion and LC-MS/MS analysis as previously reported.[4] All peptide samples were dried in speedvac and reconstituted with 20 μL of 0.1% TFA/H2O. LC-MS analysis was performed on a nanoAcquity (Waters, inc) configured with Q-exactive Orbitrap mass spectrometer (ThermoScientific Inc.). Digests were loaded onto a fused silica desalting column packed with 2 cm C18 reverse phase resin (Phenomenex Inc.), and peptides were eluted on a reverse phase column (100 μm fused silica packed with 25 cm C18 resin) using a gradient 5-50% Buffer B in Buffer A (Buffer A: water, 0.1 % formic acid; Buffer B: acetonitrile, 0.1% formic acid). The flow rate though the column was set to 250 nl min-1and spray voltage was set to 2.0 kV. One full MS scan (350 – 1800 MW) was followed by 10 data dependent scans of most intense ions with dynamic exclusion enabled. The generated tandem MS data was searched using Uniprot (Taxonomy: Human, 2017.04.11) using Proteome Discoverer software (v.1.4.0.288). A maximum of three missed cleavages were allowed, and fully tryptic peptides were considered with dynamic modification of acetyl at *N*-termini. The maximal mass tolerance in MS mode was set to 10 ppm, and fragment MS/MS tolerance was set to 0.8 Da for HCD data. The maximum peptide and site false discovery rates were specified as 0.01 using Percolator. The obtained peptide/protein list for each fraction was exported to Microsoft Excel or processed using an in-house script for further analysis. ID mapping feature of the UniProt Database (http://www.uniprot.org/uploadlists/) was used to determine the localization and function of the proteins.

4. Results

4.1 Cleaved Fragments of L1 Confirmed By LCMS

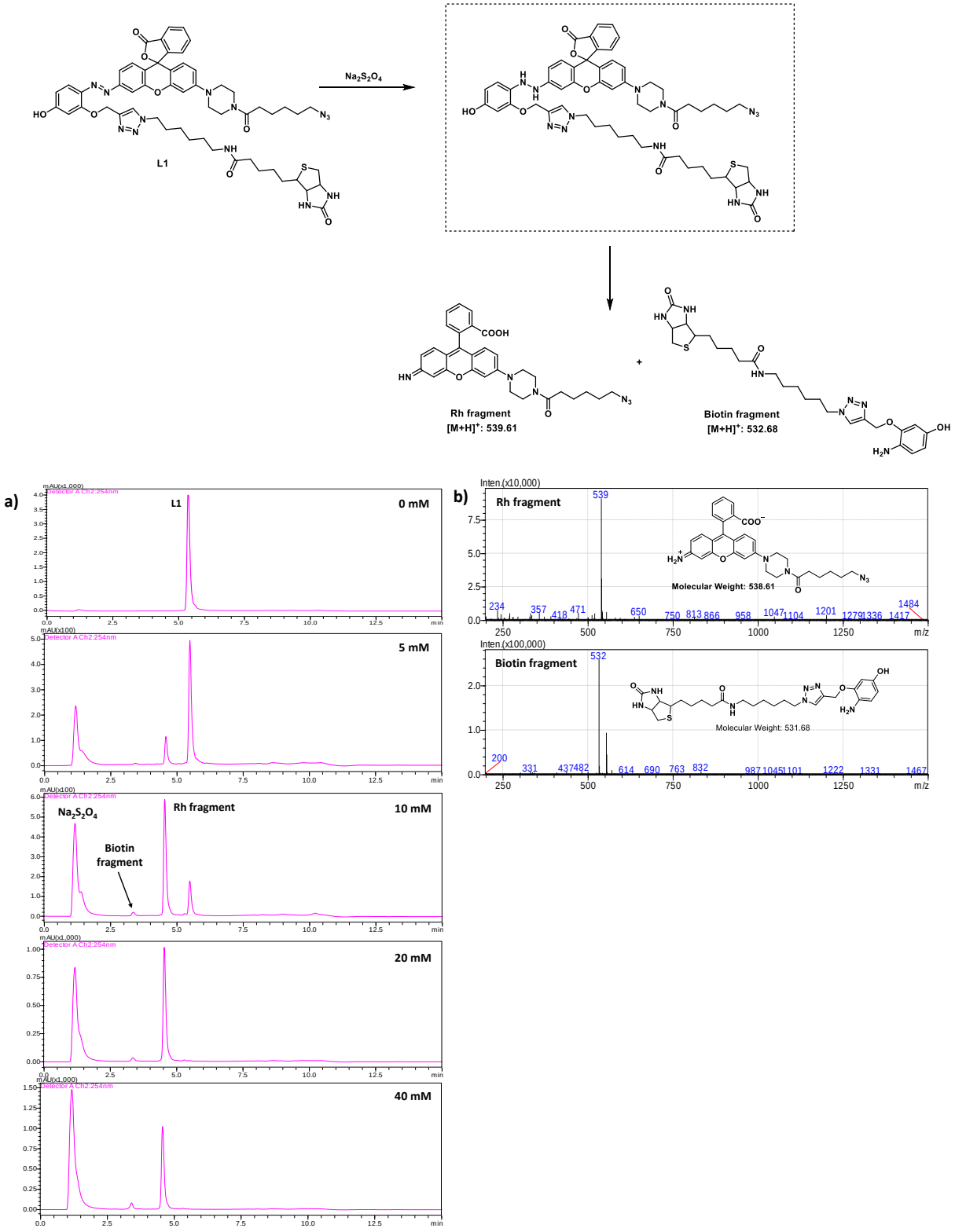


*Figure S2. a) 50 μM of **L1** was cleaved using 0, 5, 10, 20 and 40 mM of $Na_2S_2O_4$ in PBS (pH 7.4). LC-MS was used to analyse the reactions after 30 mins. b) LCMS confirmed the m/z of the Rh fragment and Biotin fragment. The partially-cleaved hydrazine intermediate was not observed.*

4.2 Determination of L1's Click Reaction Efficiency *In-vitro*

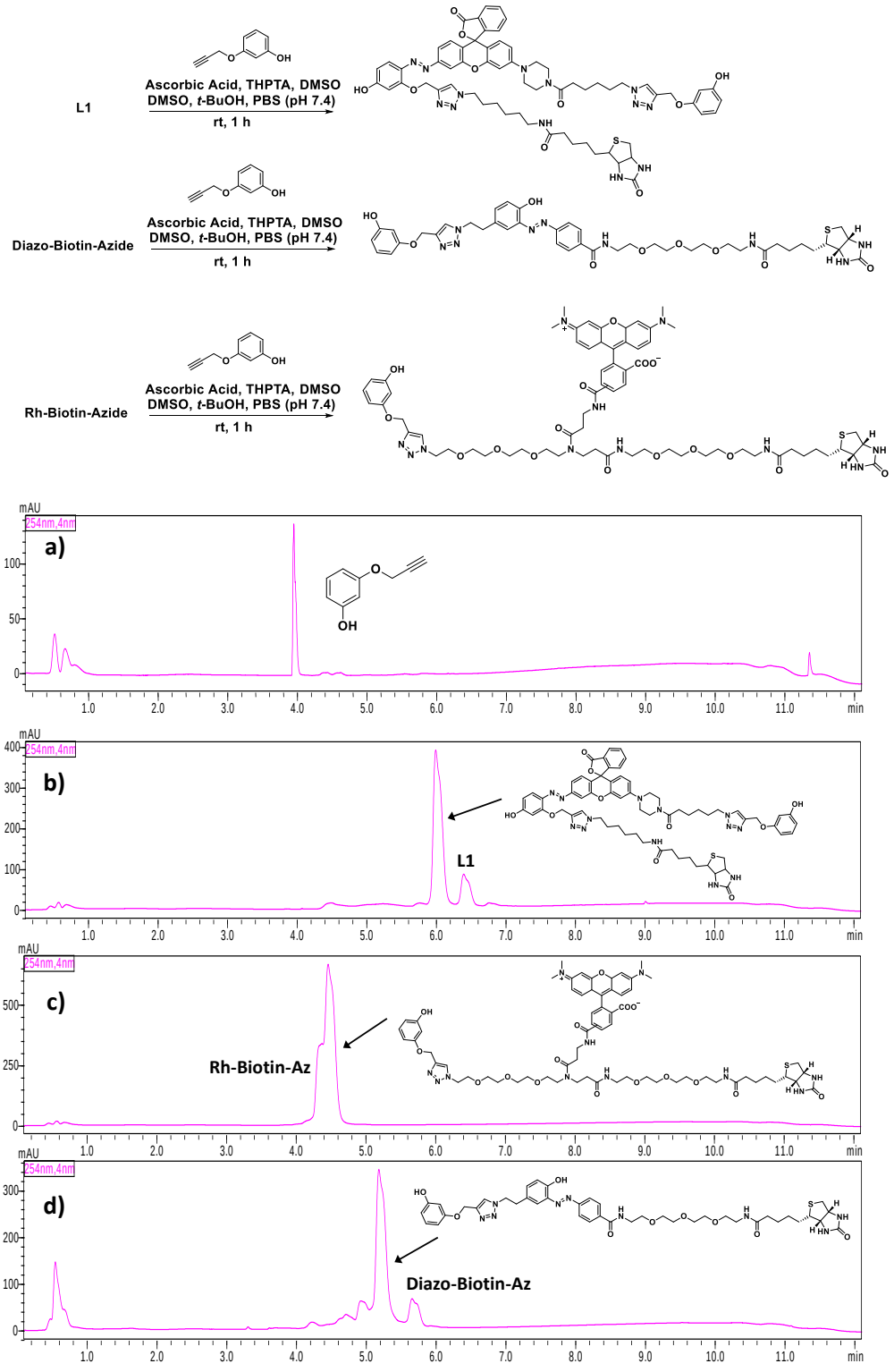


*Figure S3. Click reaction was carried out with 3-propargyloxyphenol (1.0 equiv), linker (1.5 equiv), sodium ascorbate (6.0 equiv), THPTA (2 .0 equiv) and $CuSO_4$ (2.0 equiv) in DMSO/t-BuOH and PBS (pH 7.4). The reaction was shaken at room temperature and monitored every 1 h using LCMS. a) Negative control: Sodium ascorbate + THPTA + $CuSO_4$ + 3-propargyloxyphenol b) Click reaction of **L1** with 3-propargyloxyphenol c) Click reaction of Rh-Biotin-Az with 3-propargyloxyphenol d) Click reaction of*

*Diazo-Biotin-Az with 3-propargyloxyphenol. After 1 h incubation, b), c) and d) indicated that 3-propargyloxyphenol (limiting reagent) was completely consumed. The desired triazole products were formed with all three linkers. These results showed that **L1** displayed similar click efficiency when compared to Diazo-Biotin-Az and Rh-Biotin-Az.*

4.3 Optimisation of L1's Concentration In PDI System

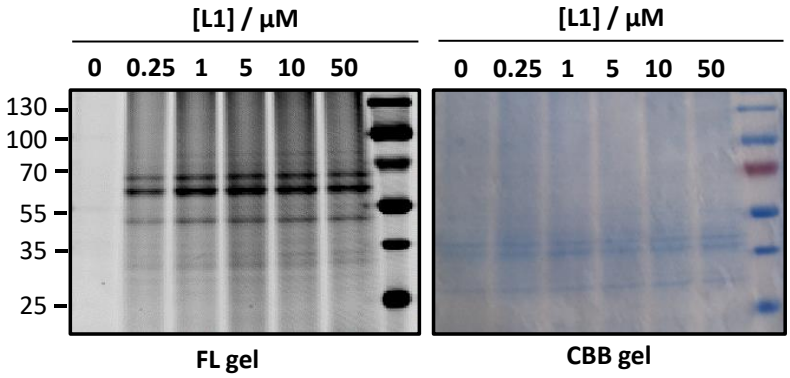


*Figure S4. MCF-7 cells in situ labeled with 5 µM **P1** were lysed and clicked with 0, 0.25, 1, 5, 10, 50 µM of **L1**. After acetone precipitation, the labeled proteome lysate was resolubilised in 1% SDS, cleavage was carried out with 100 mM of freshly prepared $Na_2S_2O_4$ solution (in PBS), separated by SDS-PAGE gel followed by direct in-gel fluorescence scanning. The intensity of the 57-kDa fluorescent bands (corresponding to human PDI) reached saturation at concentrations when >1 µM **L1** was used. Even when higher concentrations of **L1** was used, no significant background labelling was observed. Hence, 50 µM of **L1** was used for the PDI system based on established protocols from our previous work.[2]*

4.4 Optimisation of L1's Cleavage Conditions using P1

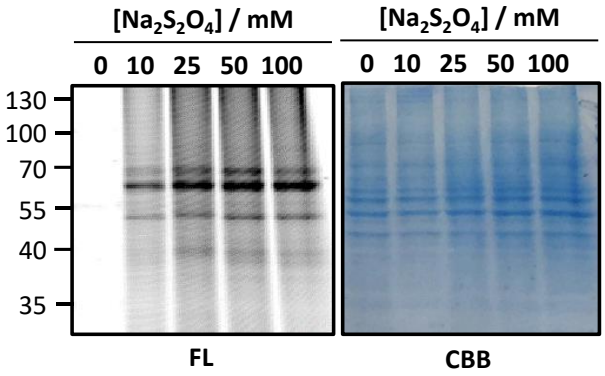


*Figure S5. MCF-7 cells in situ labeled with 5 µM **P1** were lysed and clicked with 50 µM **L1**. After acetone precipitation, the labeled proteome lysate was resolubilised in 1% SDS, cleavage was carried out with 0, 10, 25, 50 and 100 mM of freshly prepared $Na_2S_2O_4$ solution (in PBS), separated by SDS-PAGE gel followed by direct in-gel fluorescence scanning. The intensity of the 57-kDa fluorescent bands (corresponding to human PDI) reached saturation at 25 mM $Na_2S_2O_4$, indicating it was the lowest optimised cleavage concentration for subsequent experiments. (FL gel was reproduced in Fig. 3B)*

4.5 PDI Pull-down (PD)

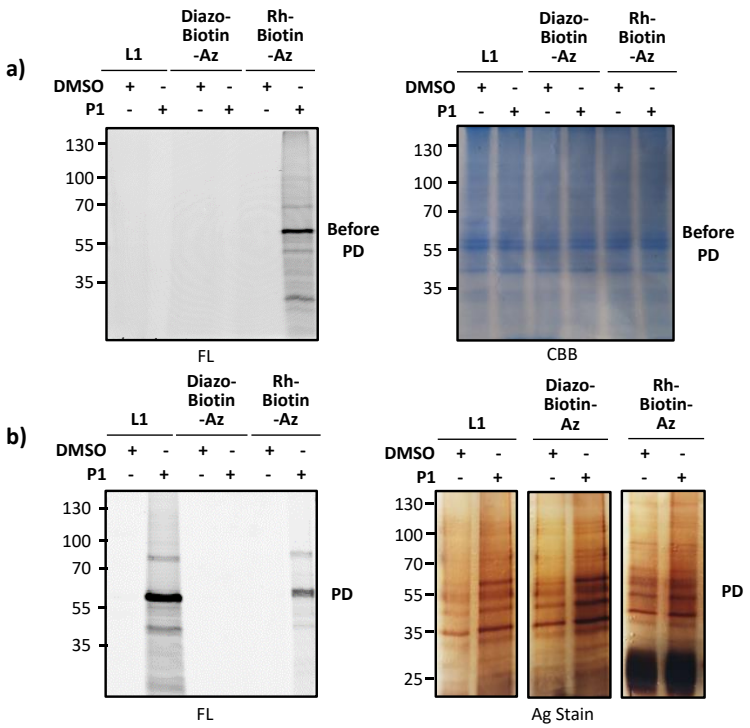


Figure S6. ***P1*** *treated MCF-7 cells were lysed and clicked with 50 µM* ***L1,*** *Diazo-Biotin-Az and Rh-Biotin-Az. After acetone precipitation, the labeled proteome lysate was enriched using Neutravidin™ beads. Proteins pull-downed by* ***L1*** *and Diazo-Biotin-Az were eluted using 25 mM of $Na_2S_2O_4$ in 1% SDS (3 x 15 min). Samples pull-downed using Rh-Biotin-Azide were boiled with 2 x standard SDS-loading buffer for 30 min at 95°C with gentle mixing. The combined eluates of* ***L1*** *and Diazo-Biotin-Az and bead bound fractions from Rh-Biotin-Azide were then combined and loaded onto 12% SDS PAGE gel, resolved by electrophoresis and visualized by in-gel fluorescence scanning/silver stain. a) Fluorescence and CBB gel of* ***P1****-labelled lysate clicked with all three linkers (before pull-down). No fluorescence was observed for the* ***L1*** *sample before reductive cleavage. b) Fluorescence and silver stain gel of total protein after pull-down and cleavage with the three linkers. Fluorescence turn-on was observed for the* ***L1*** *sample after reductive cleavage. In contrast with Rh-Biotin-Az, contamination from avidin monomers (~20 kDa region) after boiling of beads were not present in the* ***L1*** *and Diazo-Biotin-Az samples.*

4.6 PDI LC-MS/MS Analysis

Table S1. MCF-7 cells in situ labeled with 5 µM ***P1*** *for 4 h were lysed and clicked with 50 µM* ***L1****, Diazo-Biotin-Az and Rh-Biotin-Az via CuAAC for 2 h. After enrichment using Neutravidin™ beads, the bound protein was eluted (3 x 15 min) with 25 mM $Na_2S_2O_4$ (dissolved in PBS buffer with 1% SDS). The eluates were combined, separated by 12% SDS-PAGE gel and subjected to in-gel fluorescence scanning as well as CBB staining. The strongly fluorescently-labeled/CBB-stained bands around the region of ~57 kDa were excised from the gel. The proteins were subjected to tryptic in-gel digest digestion as previously reported and LC-MS/MS analysis was carried out.[4] All proteins identified with molecular weights cut-off at 57 kDa were extracted from the raw data and tabulated below. PDI was successfully identified (red) by all three linkers alongside with the respective background proteins (grey) as less significant hits.*

Description	MW [kDa]	Score	# Peptides	emPAI
L1				
Protein disulfide-isomerase	**57.081**	**7.27889812**	**3**	**0.189**
Pyruvate kinase	57.9	3.785791874	1	0.064
Diazo-Biotin-Az				
Protein disulfide-isomerase	**57.081**	**44.01720262**	**4**	**0.259**
Heat shock factor protein 1	57.225	3.740122199	1	0.116
Hydroxymethylglutaryl-CoA synthase, cytoplasmic	57.257	1.85627532	2	0.292
Cortactin, isoform CRA_c	57.432	4.466739416	2	0.186
cDNA FLJ51385, highly similar to Ran GTPase-activating protein 1	57.722	11.23547637	2	0.186
Testicular tissue protein Li 192	57.1	0	1	0.059
BTB (POZ) domain containing 14B, isoform CRA_a	57.222	0	1	0.089
Phosphoribosyl pyrophosphate amidotransferase proprotein variant (Fragment)	57.348	0	1	0.08
cDNA FLJ45688 fis, clone FCBBF3021191, highly similar to Protein phosphatase 2C isoform gamma (EC 3.1.3.16)	57.355	0	1	0.093
Amidophosphoribosyltransferase	57.362	0	1	0.08
Protein LYRIC	57.487	0	1	0.086
Acetyltransferase component of pyruvate dehydrogenase complex	57.55	0	1	0.083
Serine/theonine-protein phosphatase (Fragment)	57.622	0	1	0.083
Heterogeneous nuclear ribonucleoprotein U (Scaffold attachment factor A), isoform CRA_a	57.627	0	2	0.133
Aldehyde dehydrogenase 6A1 variant (Fragment)	57.789	0	1	0.07
Importin subunit alpha	57.798	0	2	0.172
Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	57.803	0	1	0.07
Importin subunit alpha-1	57.826	0	2	0.172
Importin subunit alpha	57.84	0	2	0.172
Epithelial-splicing regulatory protein 1 (Fragment)	57.87	0	1	0.077
Importin subunit alpha	57.929	0	2	0.166
Rh-Biotin-Azide				
Protein disulfide-isomerase	**57.081**	**24.74976635**	**6**	**0.496**

cDNA FLJ51385, highly similar to Ran GTPase-activating protein 1	57.722	1.601182461	1	0.089
Heat shock factor protein 1	57.225	0	1	0.116
Putative uncharacterised protein DKFZp781C0423 (Fragment)	57.409	0	1	0.089
cDNA, FLJ95176, Homo sapiens CGI-48 protein (CGI-48), mRNA	57.671	0	1	0.086

4.7 HPG LC-MS/MS Analysis

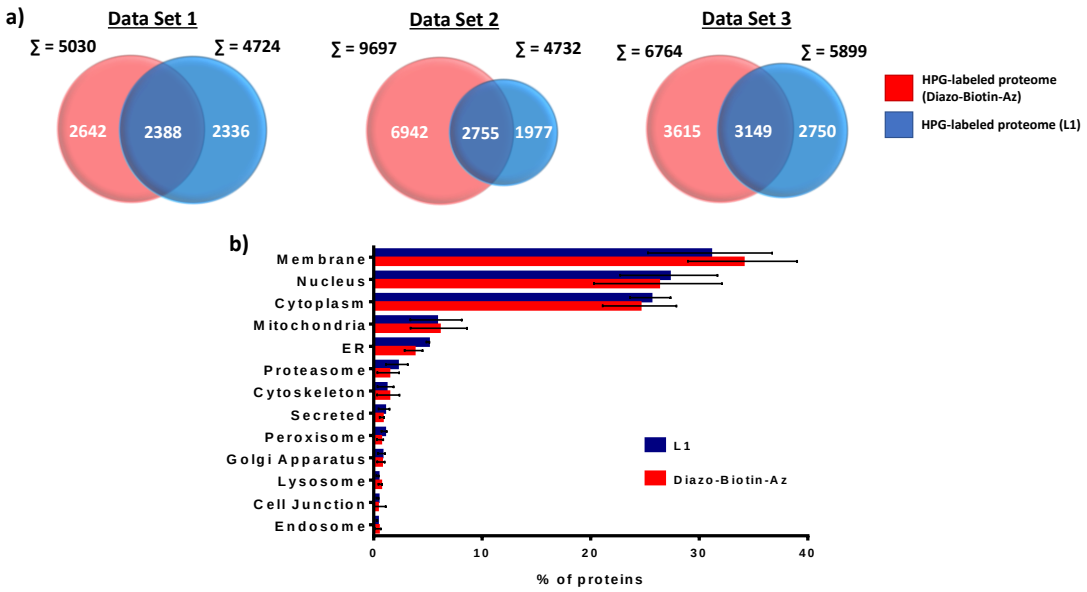


*Figure S7. HeLa cells in situ labeled with 100 µM L-HPG for 8 h were lysed and clicked with 50 µM **L1**, Diazo-Biotin-Az and Rh-Biotin-Az via CuAAC for 2 h. After enrichment using Neutravidin™ beads, the bound protein was eluted (3 x 15 min) with 25 mM $Na_2S_2O_4$ (dissolved in PBS buffer with 1% SDS). The eluates were combined, separated by 12% SDS-PAGE gel and subjected to in-gel fluorescence scanning as well as CBB staining. The strongly fluorescently-labeled/CBB-stained bands were excised from the gel. The proteins were subjected to tryptic in-gel digest digestion as previously reported and LC-MS/MS analysis was carried out.[4] a) In this proteomics study, triplicate analysis was carried out (raw data can be found in the Supplementary Excel file). The HPG-labeled proteome enriched by **L1** (blue) and Diazo-Biotin-Az (red) in each triplicate are represented in data sets 1, 2 and 3 respectively. Total number of proteins pulled-down by each linker was also indicated. Based on the triplicates, data set 2 was not analyzed further because of severe discrepancies when compared to data set 1 & 3. Fig. 4B was derived from data set 1 and 3. Proteins identified by **L1** and Diazo-Biotin-Az, appearing in both data sets 1 and 3 were picked out and tabulated in Fig. 4B. b) Sub-cellular localization of newly synthesised proteomes captured by **L1** (blue) and Diazo-Biotin-Az (red) obtained from data set 1 and 3 with error bars (reproduced in Fig. 4C).*

5. Characterisation

5.1 H-MS Analysis

H-MS (ESI) OF C2

Mass Spectrum SmartFormula Report

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Comment	Prof. Yao Shao Qin		

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Scan End	1800 m/z	Set Collision Cell RF	200.0 Vpp	Set Divert Valve	Waste

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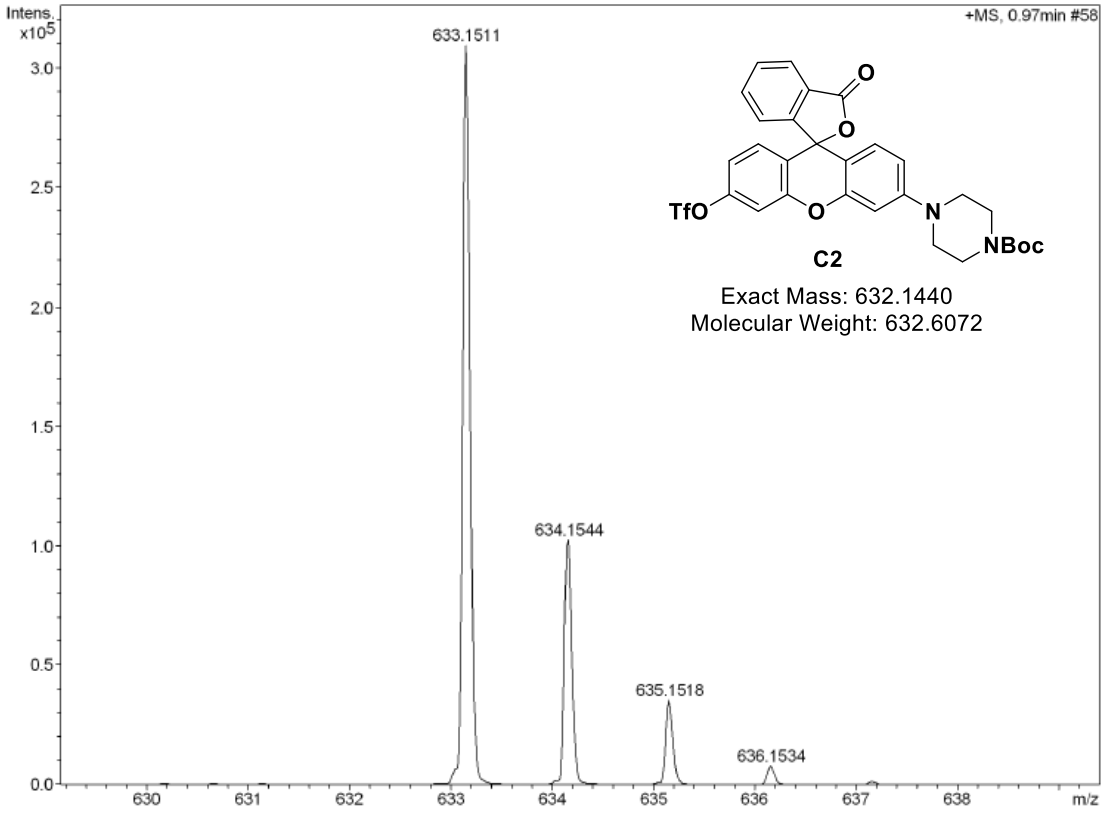

Bruker Compass DataAnalysis 4.0 printed: 11/15/2016 4:47:41 PM Page 1 of 1

H-MS (ESI) OF C3

Mass Spectrum SmartFormula Report

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Comment	Prof. Yao Shao Qin		

Acquisition Parameter

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Meas. m/z	#	Formula	m/z	err [ppm]	rdb	e¯ Conf	N-Rule
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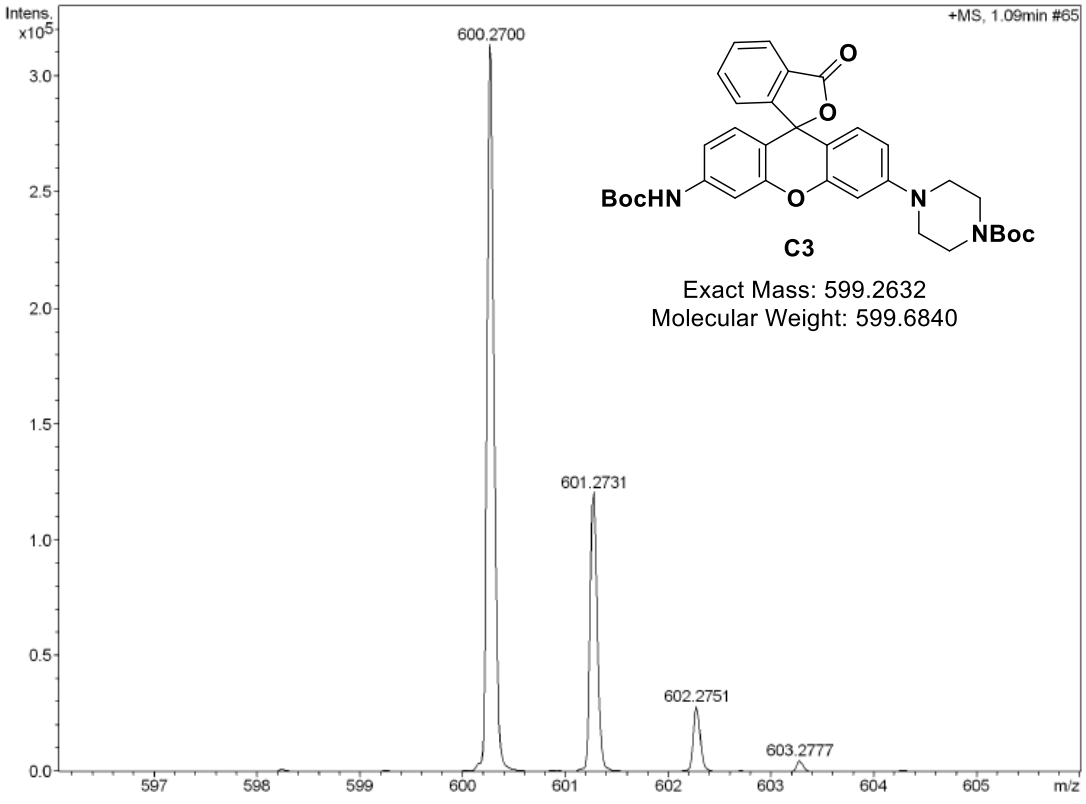

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H-MS (ESI) OF C5

Mass Spectrum SmartFormula Report

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Comment	Prof. Yao Shao Qin		

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Meas. m/z	#	Formula	m/z	err [ppm]	rdb	e⁻ Conf	N-Rule
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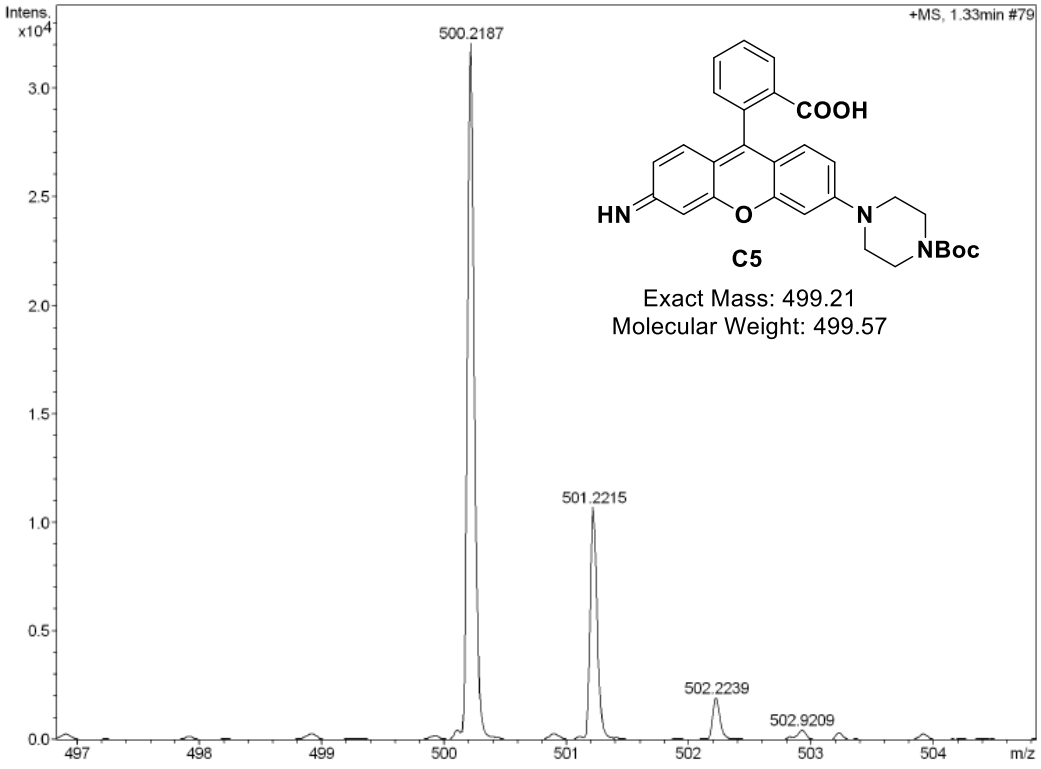

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H-MS (ESI) OF C6

Mass Spectrum SmartFormula Report

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Comment	Prof Yao S Q		

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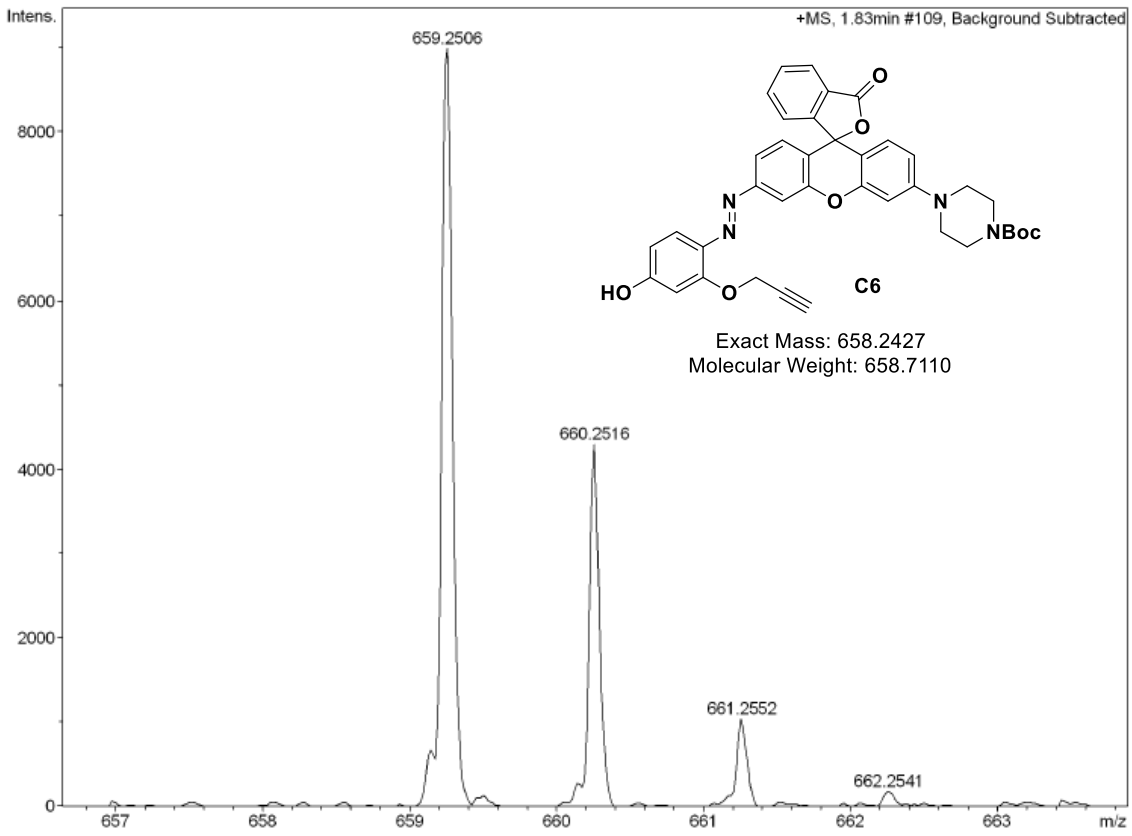

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H-MS (ESI) OF C7

Mass Spectrum SmartFormula Report

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Comment	Prof Yao S Q		

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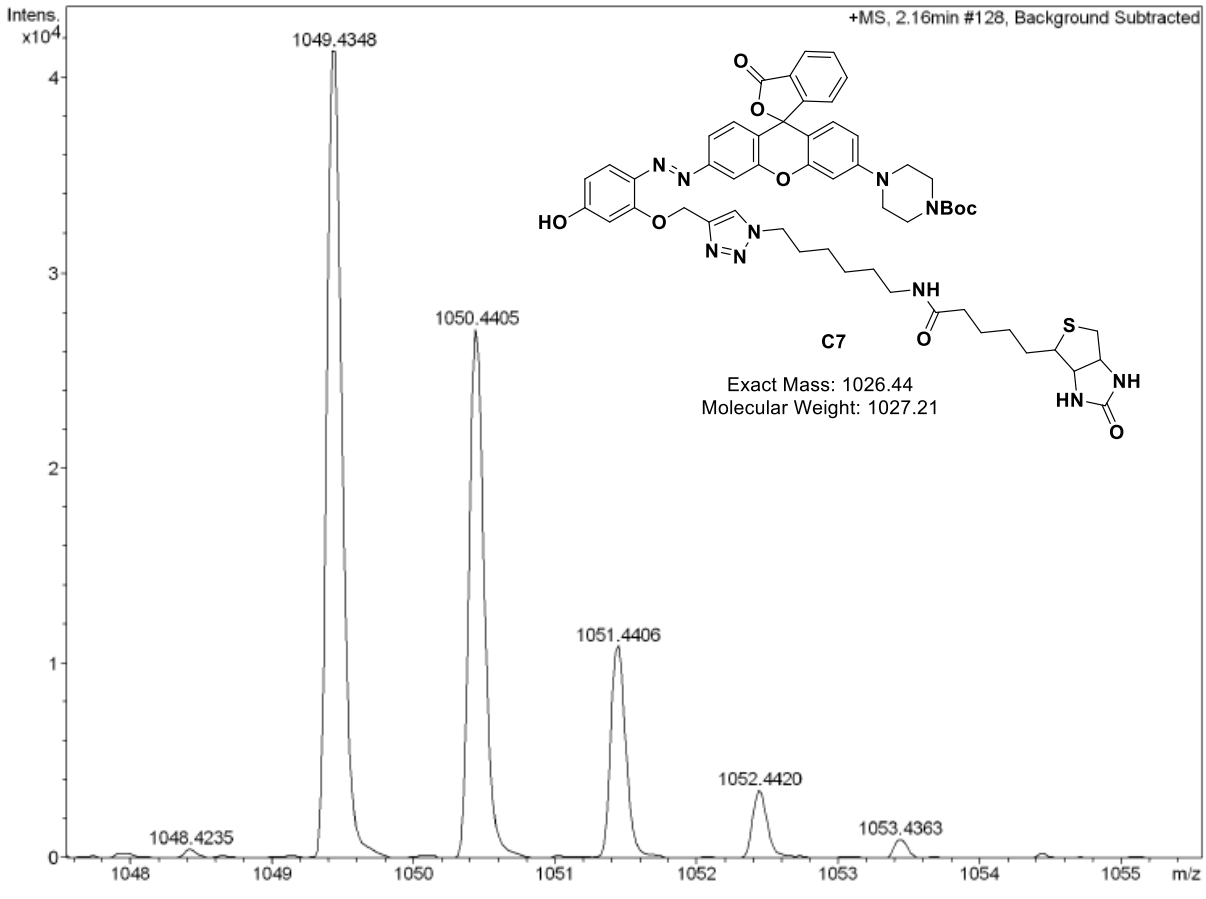

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H-MS (ESI) OF P1

Mass Spectrum SmartFormula Report

Analysis Info

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Comment	Prof. Yao Shao Qin		

Acquisition Parameter

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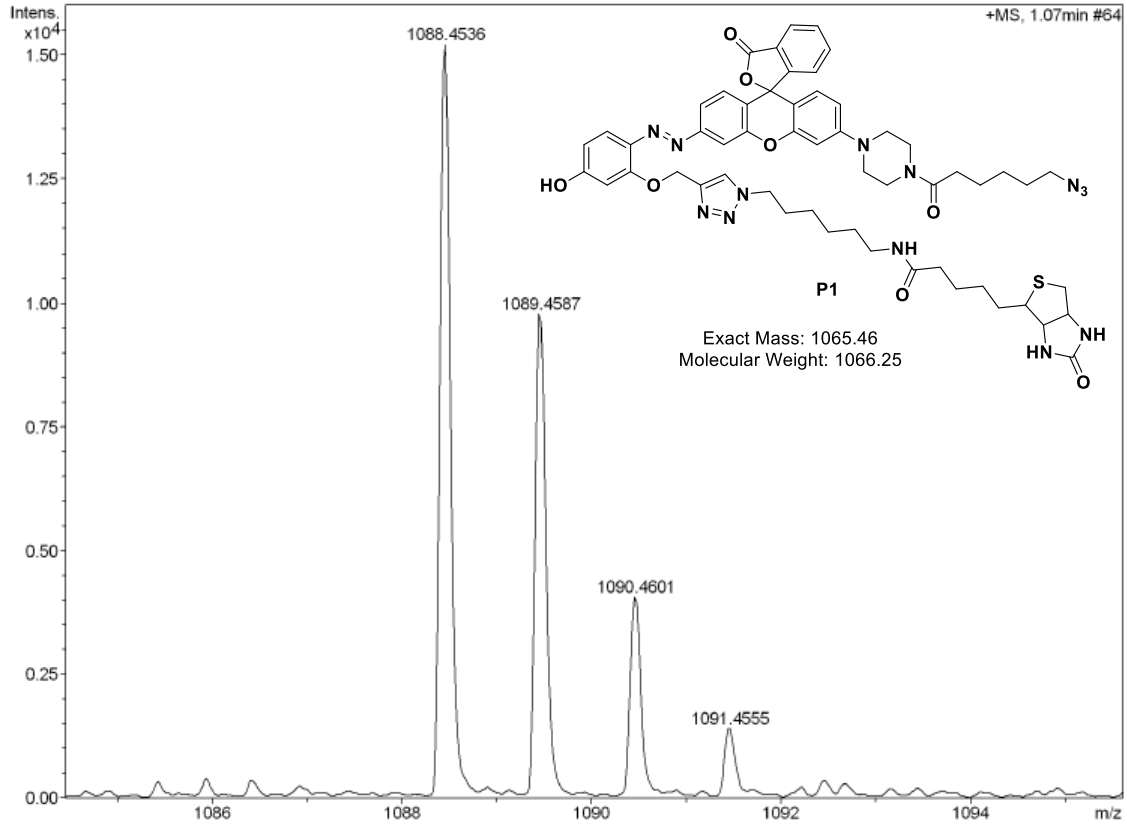

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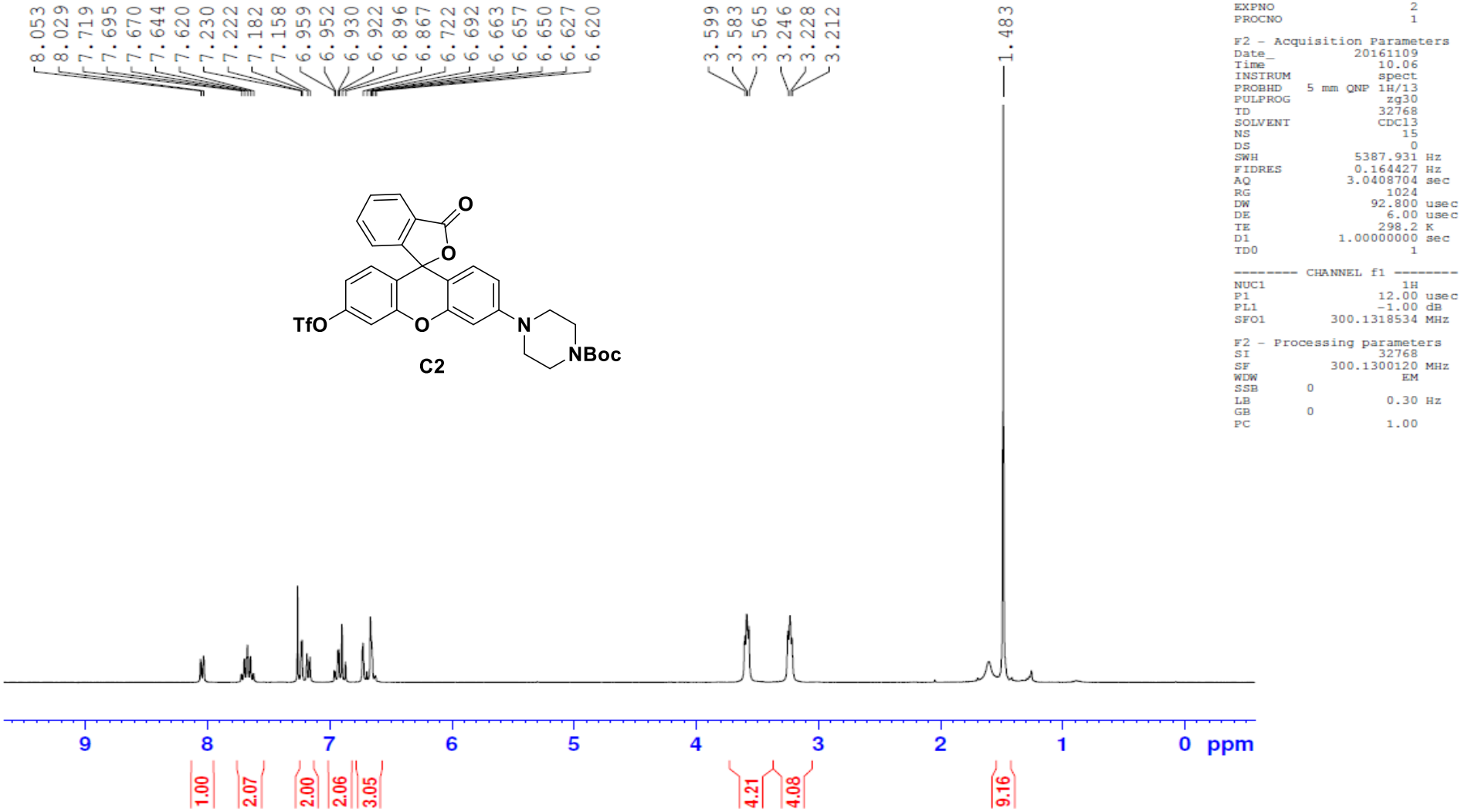
5.2 ^{1}H & ^{13}C NMR

^{1}H NMR OF C2

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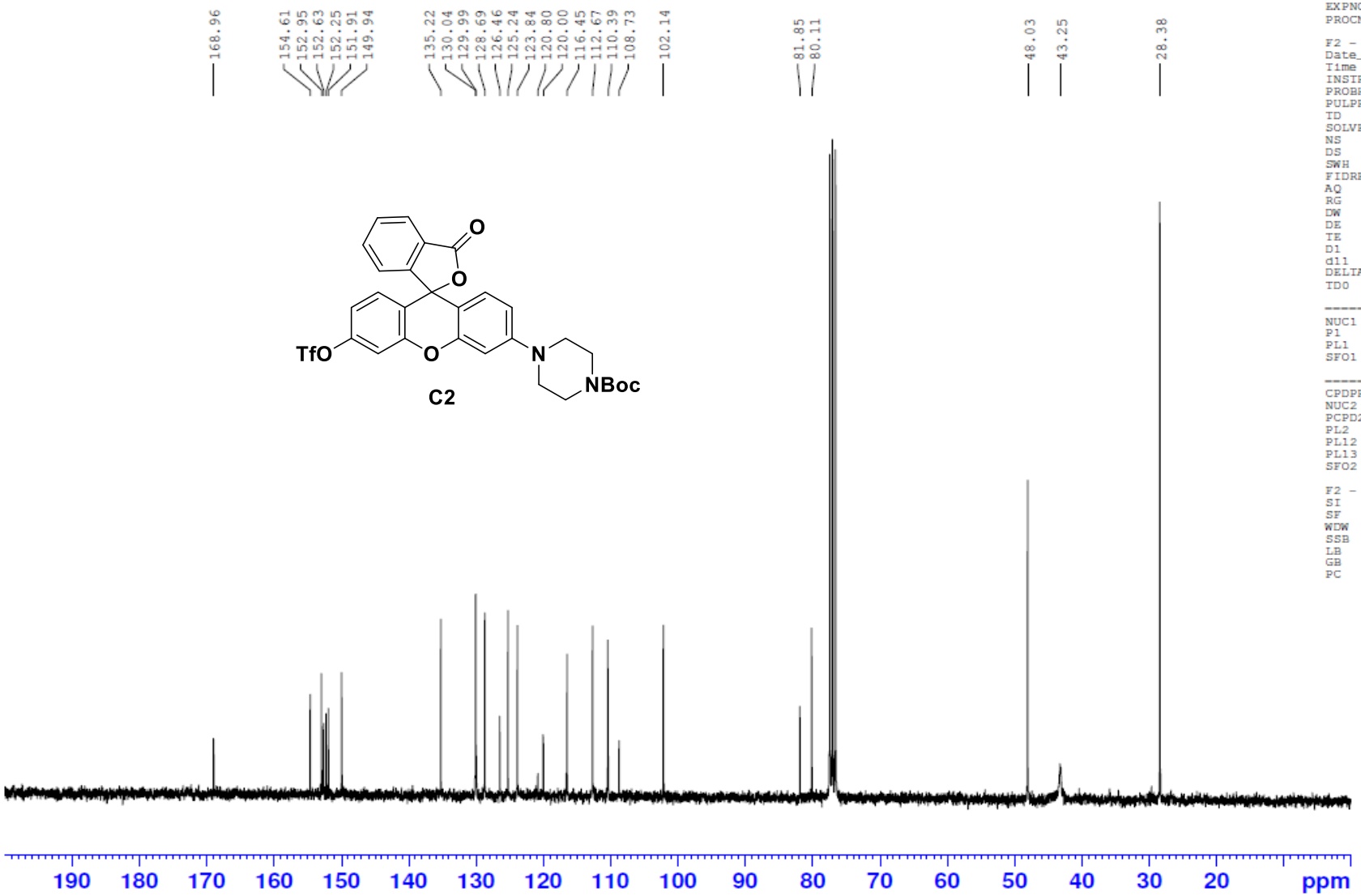
DS                           0
SWH                5387.931 Hz
FIDRES             0.164427 Hz
AQ                 3.0408704 sec
RG                        1024
DW                   92.800 usec
DE                    6.00 usec
TE                    298.2 K
D1                 1.00000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                        1H
P1                    12.00 usec
PL1                   -1.00 dB
SFO1           300.1318534 MHz

F2 - Processing parameters
SI                       32768
SF             300.1300120 MHz
WDW                         EM
SSB          0
LB                    0.30 Hz
GB           0
PC                    1.00
```

^{13}C NMR OF C2




```
Current Data Parameters
NAME                  novl1sis1
EXPNO                         3
PROCNO                        1

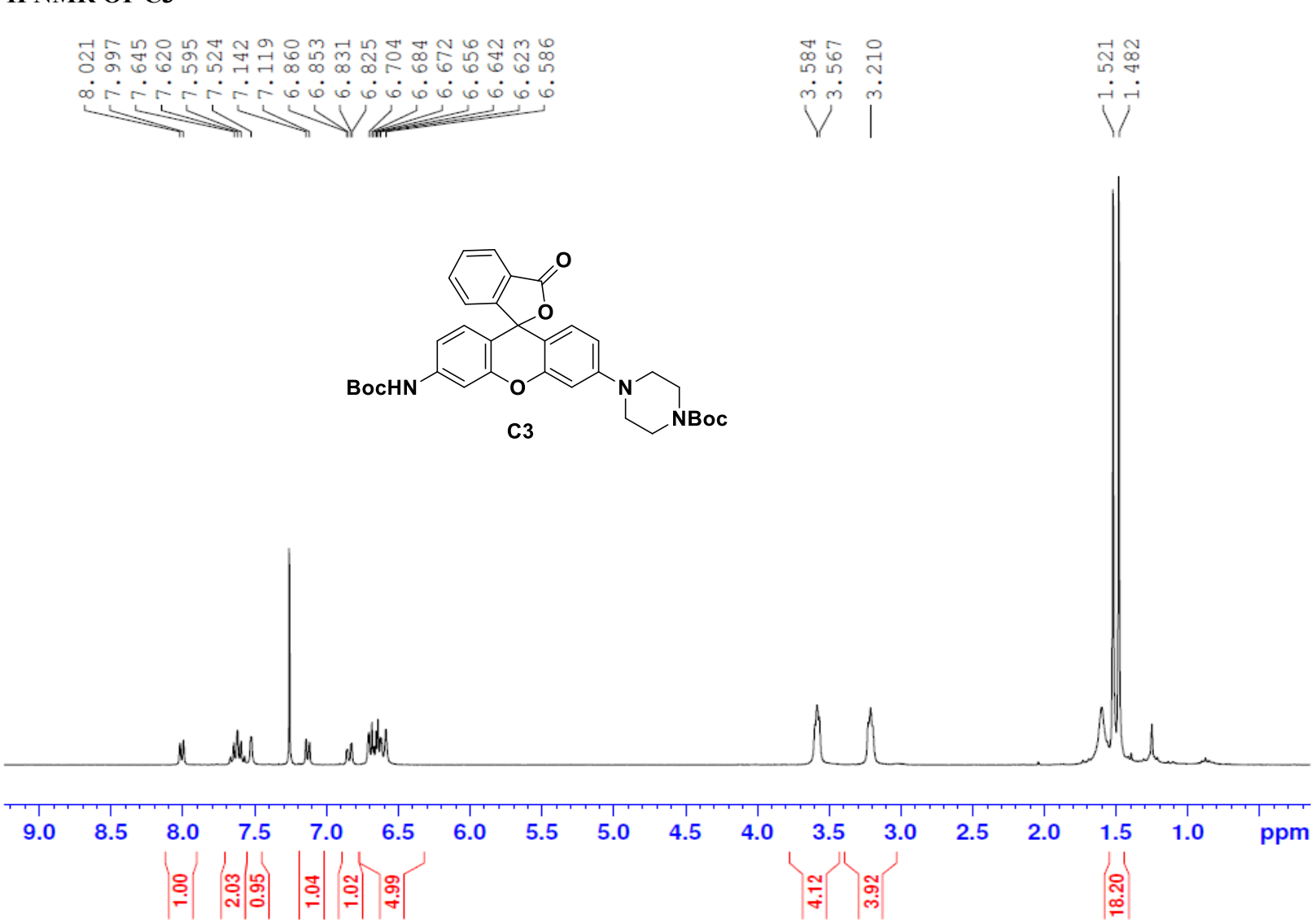
F2 - Acquisition Parameters
Date_                  20161111
Time                       9.11
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                 zgpg30
TD                         32768
SOLVENT                  CDC13
NS                          1437
DS                             2
SWH                   17985.611 Hz
FIDRES                0.548877 Hz
AQ                    0.9109504 sec
RG                         32768
DW                       27.800 usec
DE                        6.00 usec
TE                       301.4 K
D1                    1.00000000 sec
d11                   0.03000000 sec
DELTA                 0.89999998 sec
TD0                            1

======== CHANNEL f1 ========
NUC1                         13C
P1                          7.80 usec
PL1                         2.00 dB
SFO1                 75.4756731 MHz

======== CHANNEL f2 ========
CPDPRG[2                waltz16
NUC2                         1H
PCPD2                      80.00 usec
PL2                        -1.00 dB
PL12                       15.48 dB
PL13                       21.00 dB
SFO2                300.1312005 MHz

F2 - Processing parameters
SI                         32768
SF                   75.4677512 MHz
WDW                           EM
SSB                  0
LB                          1.00 Hz
GB                   0
PC                          1.40
```

^{1}H NMR OF C3



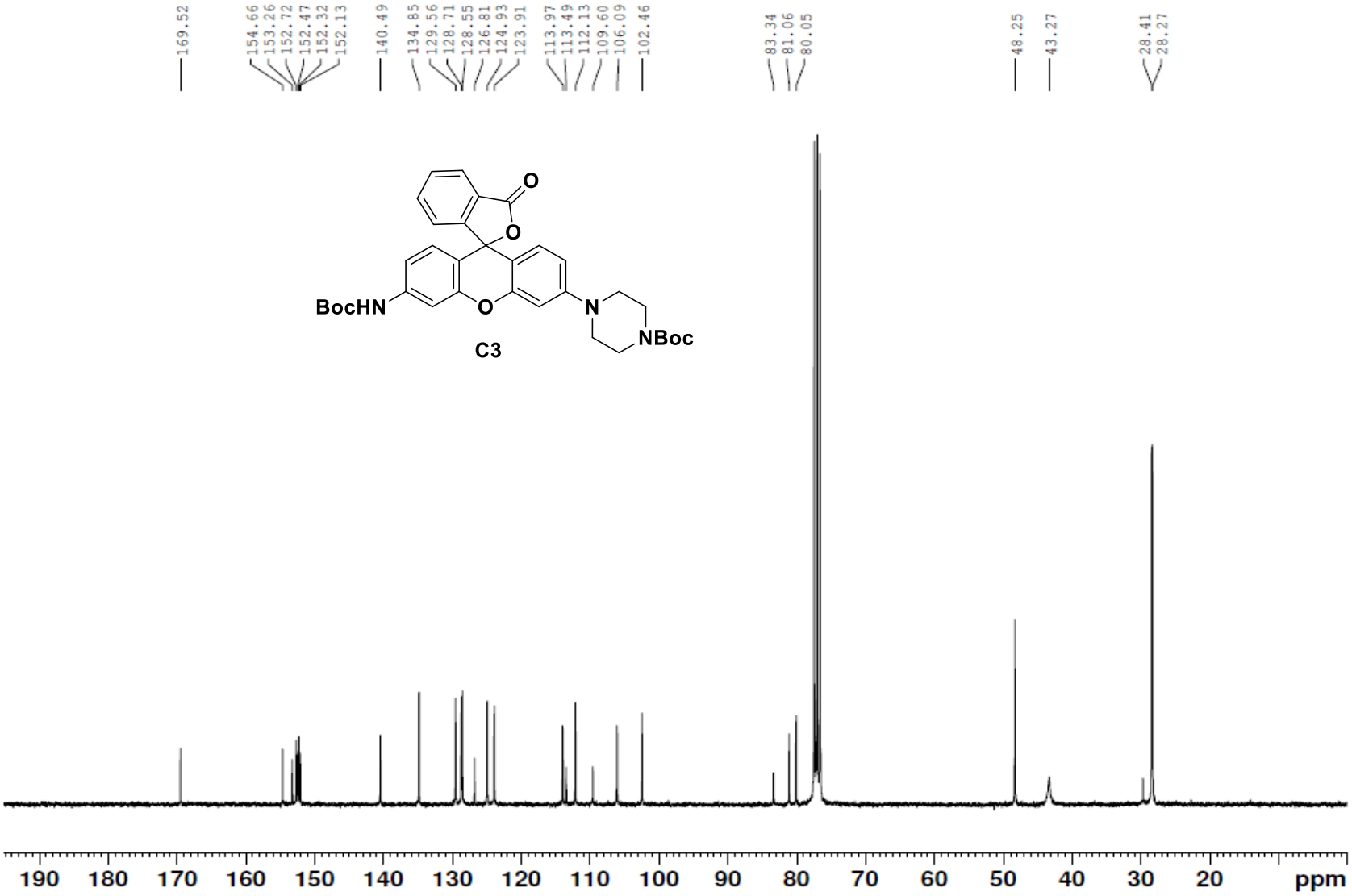

```
Current Data Parameters
NAME                  nov10sisi
EXPNO                         6
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20161110
Time                       9.29
INSTRUM                   spect
PROBHD     5 mm QNP 1H/13
PULPROG                   zg30
TD                       32768
SOLVENT                  CDC13
NS                          40
DS                           0
SWH                 5387.931 Hz
FIDRES              0.164427 Hz
AQ                  3.0408704 sec
RG                     1149.4
DW                    92.800 usec
DE                     6.00 usec
TE                     296.8 K
D1                 1.00000000 sec
TD0                          1

--------- CHANNEL f1 --------
NUC1                        1H
P1                     12.00 usec
PL1                    -1.00 dB
SFO1              300.1318534 MHz

F2 - Processing parameters
SI                       32768
SF                300.1300120 MHz
WDW                         EM
SSB          0
LB                      0.30 Hz
GB           0
PC                      1.00
```

^{13}C NMR OF C3




```
Current Data Parameters
NAME                  nov22sisi
EXPNO                         3
PROCNO                        1

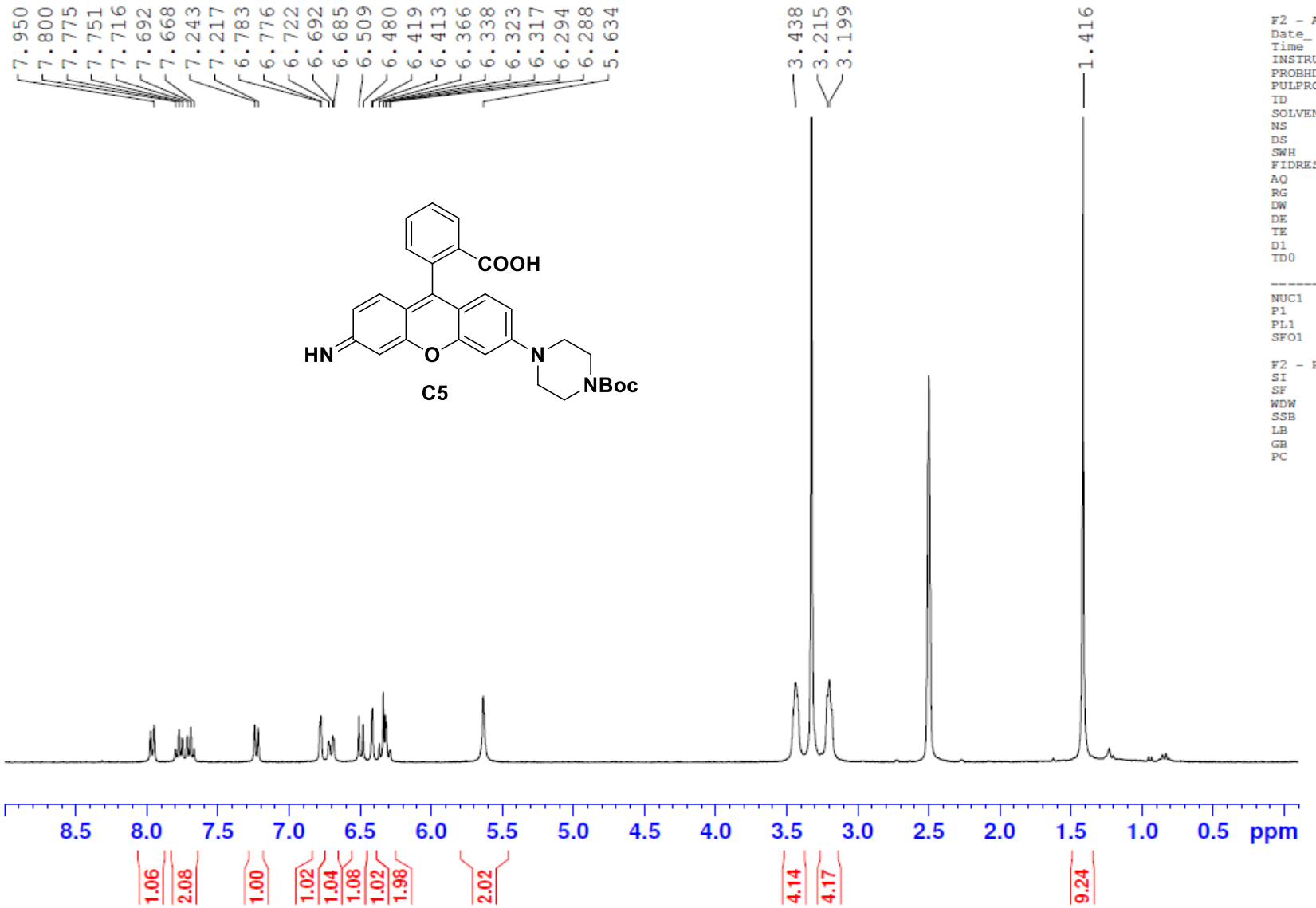
F2 - Acquisition Parameters
Date_                  20161122
Time                      21.09
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                zgpg30
TD                        32768
SOLVENT                   CDCl3
NS                        21480
DS                            2
SWH                   17985.611 Hz
FIDRES                0.548877 Hz
AQ                    0.9109504 sec
RG                        32768
DW                       27.800 usec
DE                        6.00 usec
TE                       298.4 K
D1                    1.00000000 sec
d11                   0.03000000 sec
DELTA                 0.89999998 sec
TD0                           1

======== CHANNEL f1 ========
NUC1                        13C
P1                         7.80 usec
PL1                        2.00 dB
SFO1                 75.4756731 MHz

======== CHANNEL f2 ========
CPDPRG[2                waltz16
NUC2                        1H
PCPD2                     80.00 usec
PL2                       -1.00 dB
PL12                      15.48 dB
PL13                      21.00 dB
SFO2                300.1312005 MHz

F2 - Processing parameters
SI                        32768
SF                   75.4677500 MHz
WDW                          EM
SSB                   0
LB                         1.00 Hz
GB                    0
PC                         1.40
```

^{1}H NMR OF C5



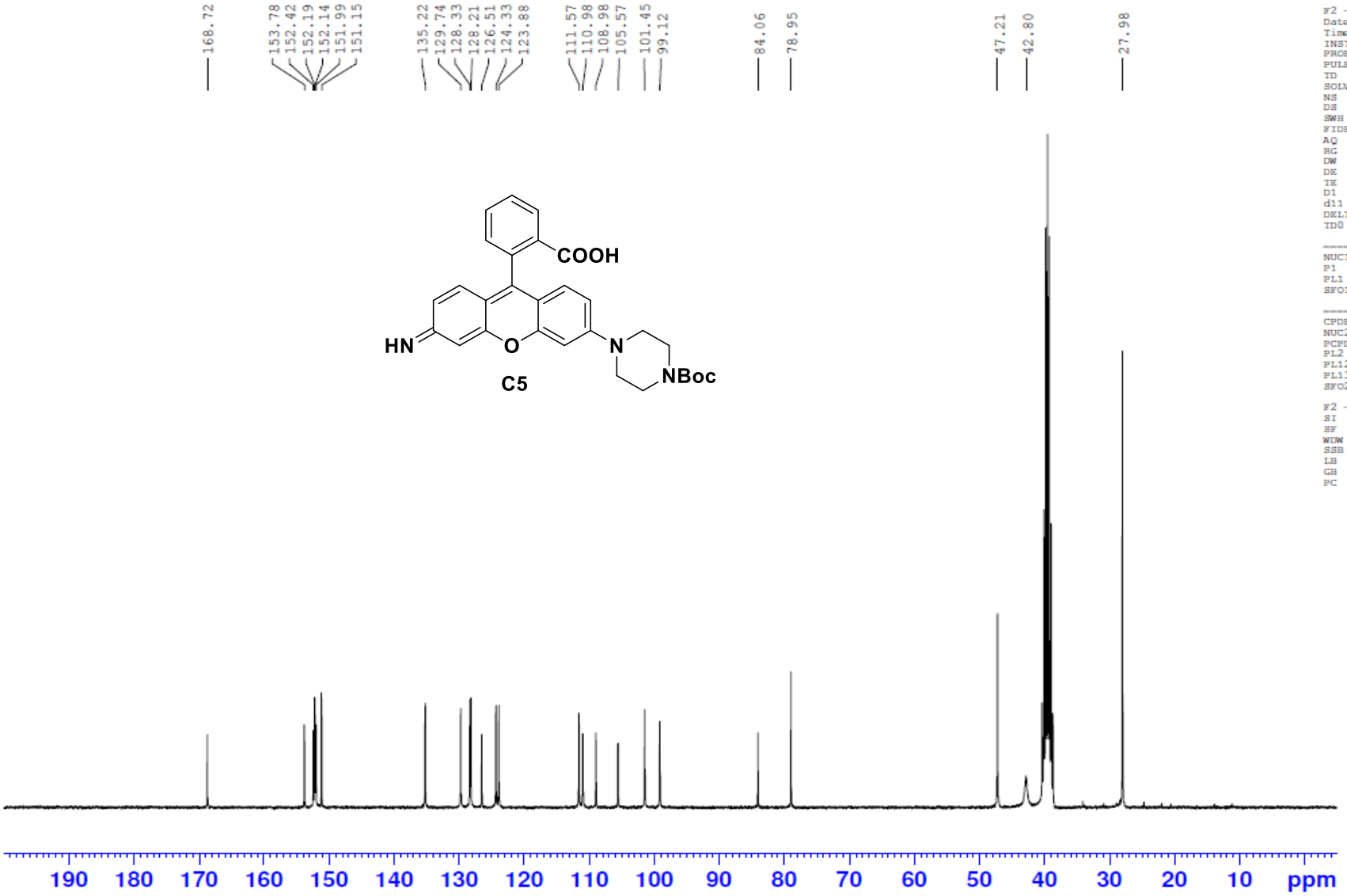

```
Current Data Parameters
NAME                  nov22sisi
EXPNO                         1
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20161122
Time                      21.01
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                   zg30
TD                       32768
SOLVENT                   DMSO
NS                          32
DS                           0
SWH                5387.931 Hz
FIDRES             0.164427 Hz
AQ                 3.0408704 sec
RG                    1290.2
DW                    92.800 usec
DE                     6.00 usec
TE                    297.3 K
D1                 1.00000000 sec
TD0                           1

-------- CHANNEL f1 --------
NUC1                         1H
P1                        12.00 usec
PL1                       -1.00 dB
SFO1              300.1318534 MHz

F2 - Processing parameters
SI                           32768
SF                300.1300000 MHz
WDW                             EM
SSB                  0
LB                        0.30 Hz
GB                   0
PC                        1.00
```

^{13}C NMR OF C5




```
Current Data Parameters
NAME            nov23sisi
EXPNO                   1
PROCNO                  1

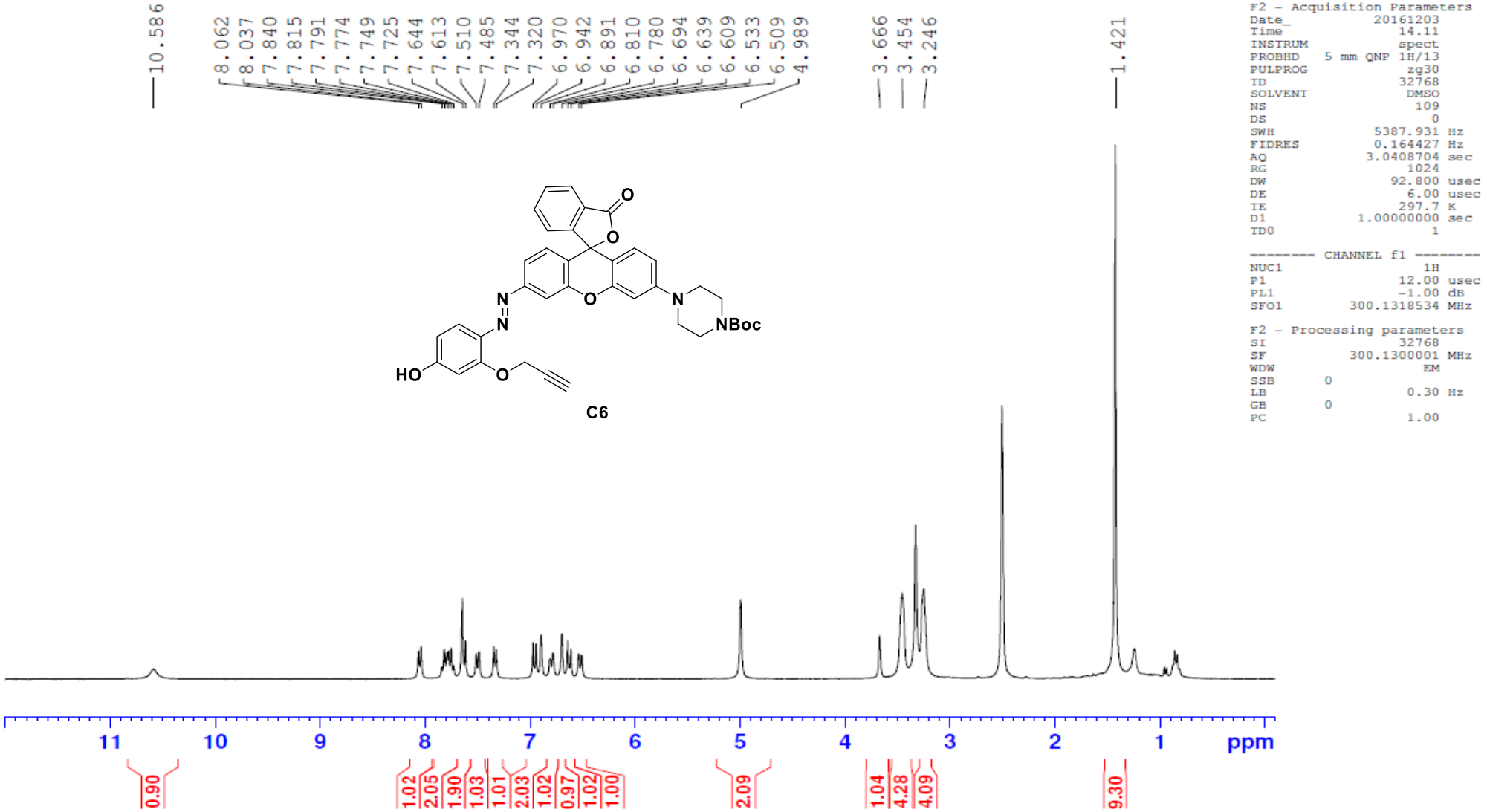
F2 - Acquisition Parameters
Date_          20161123
Time              20.01
INSTRUM           spect
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                32768
SOLVENT            DMSO
NS                22710
DS                    2
SWH           17985.611 Hz
FIDRES        0.548877 Hz
AQ            0.9109504 sec
RG                32768
DW               27.800 usec
DE                6.00 usec
TE               299.1 K
D1            1.00000000 sec
d11           0.03000000 sec
DELTA         0.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  7.80 usec
PL1                 2.00 dB
SFO1          75.4756731 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -1.00 dB
PL12               15.48 dB
PL13               21.00 dB
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                32768
SF           75.4677902 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

^{1}H NMR OF C6



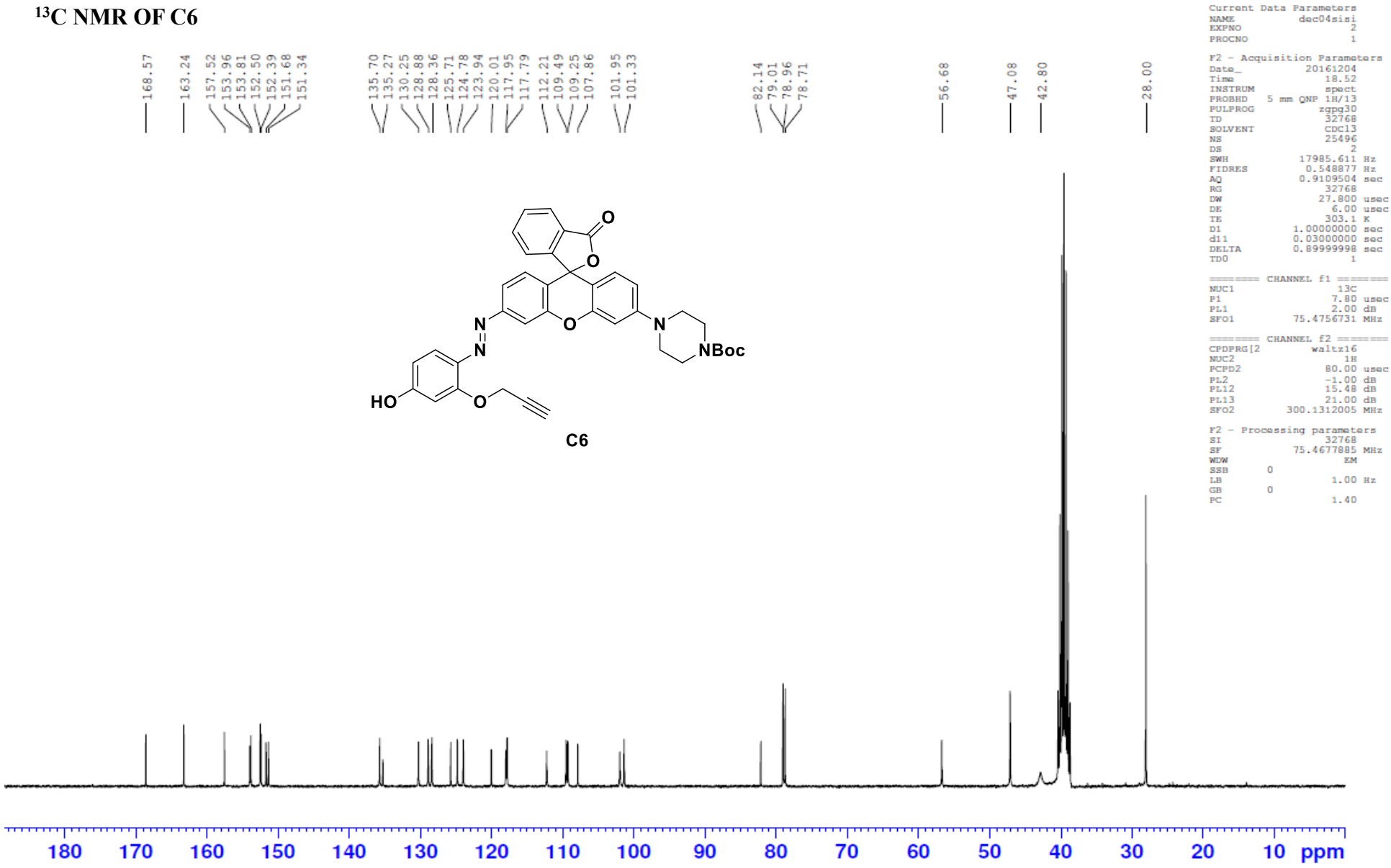

```
Current Data Parameters
NAME                 dec03sisi
EXPNO                        3
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20161203
Time                     14.11
INSTRUM                  spect
PROBHD     5 mm QNP 1H/13
PULPROG                  zg30
TD                      32768
SOLVENT                  DMSO
NS                        109
DS                          0
SWH                  5387.931 Hz
FIDRES               0.164427 Hz
AQ                   3.0408704 sec
RG                       1024
DW                     92.800 usec
DE                      6.00 usec
TE                     297.7 K
D1                  1.00000000 sec
TD0                          1

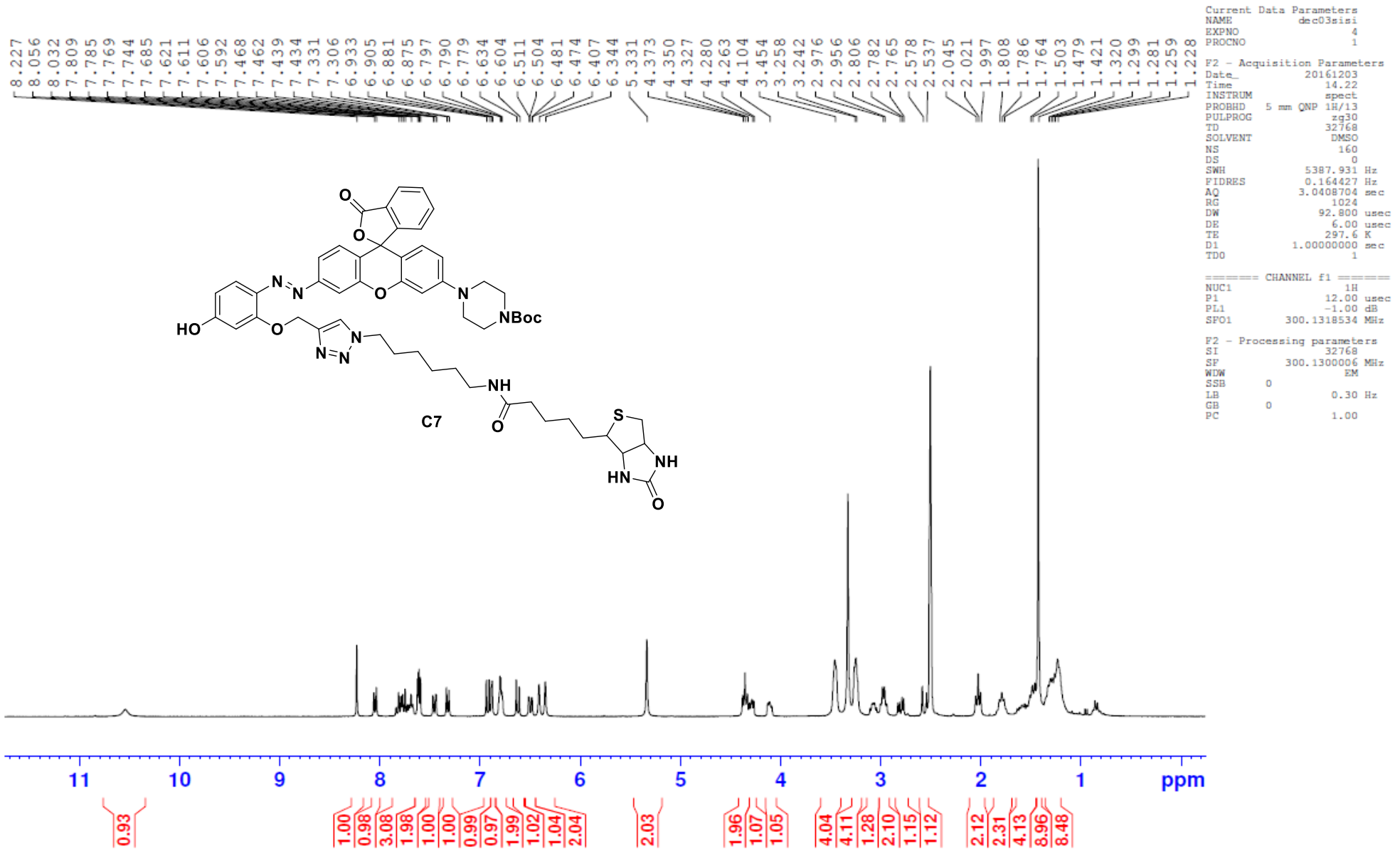
======== CHANNEL f1 ========
NUC1                        1H
P1                       12.00 usec
PL1                      -1.00 dB
SFO1               300.1318534 MHz

F2 - Processing parameters
SI                          32768
SF                 300.1300001 MHz
WDW                            EM
SSB                 0
LB                       0.30 Hz
GB                  0
PC                       1.00
```

^{13}C NMR OF C6


168.57
163.24
157.52
153.96
153.81
152.50
152.39
151.68
151.34
135.70
135.27
130.25
128.88
128.36
125.71
124.78
123.94
120.01
117.95
117.79
112.21
109.49
109.25
107.86
101.95
101.33
82.14
79.01
78.96
78.71
56.68
47.08
42.80
28.00
O
O
O
N
N
N
NBoc
HO
O
C6
Current Data Parameters
NAME dac04sisi
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161204
Time 18.52
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zqpg30
TD 32768
SOLVENT CDCl3
NS 25496
DS 2
SWH 17985.611 Hz
FIDRES 0.548877 Hz
AQ 0.9109504 sec
RG 32768
DW 27.800 usec
DE 6.00 usec
TE 303.1 K
D1 1.00000000 sec
d11 0.03000000 sec
DELTA 0.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 7.80 usec
PL1 2.00 dB
SFO1 75.4756731 MHz
======== CHANNEL f2 ========
CPDPRG[2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -1.00 dB
PL12 15.48 dB
PL13 21.00 dB
SFO2 300.1312005 MHz
F2 - Processing parameters
SI 32768
SF 75.4677885 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

^{1}H NMR OF C7



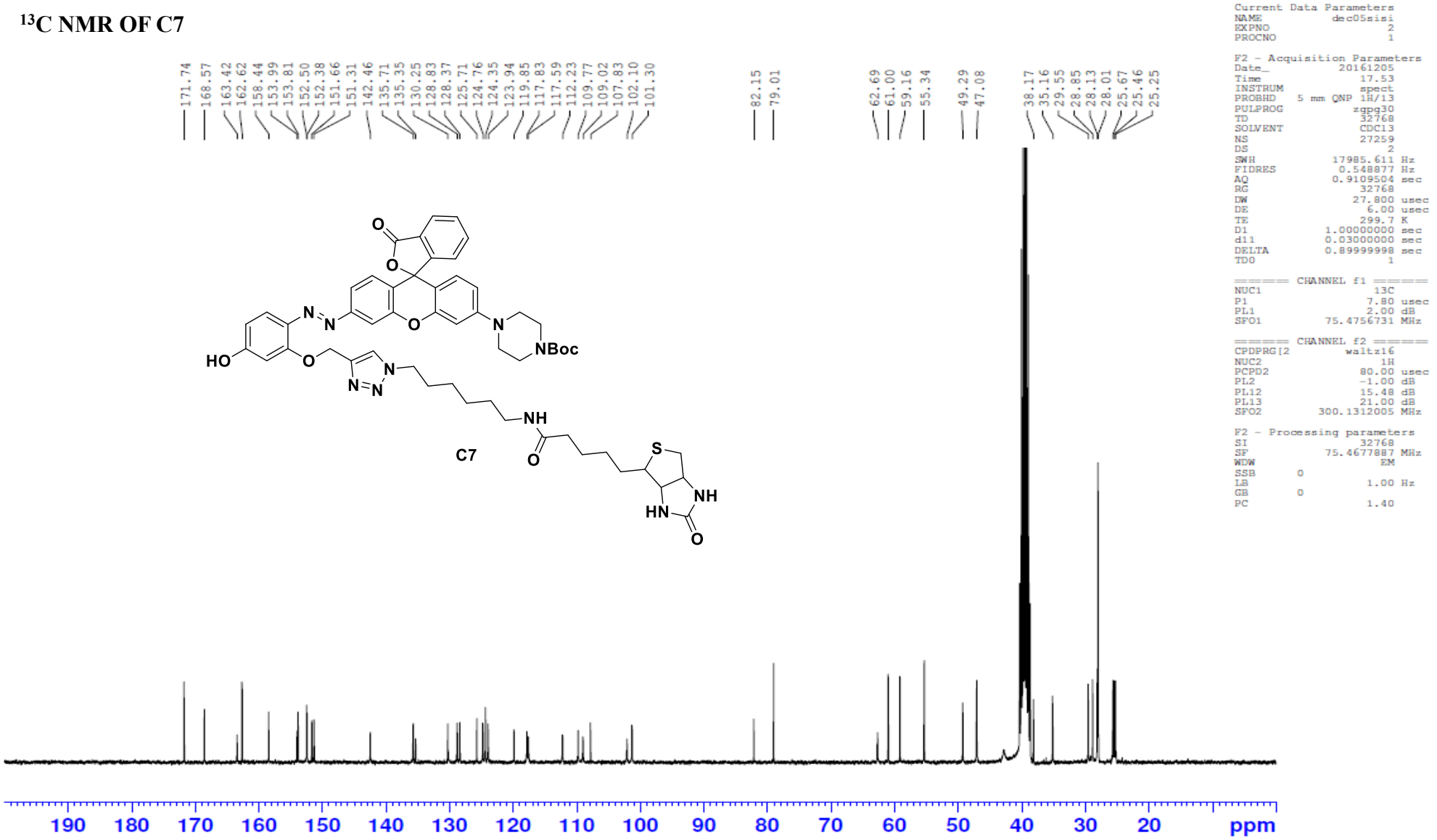

```
Current Data Parameters
NAME                  dec03sisi
EXPNO                         4
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20161203
Time                      14.22
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                   zg30
TD                       32768
SOLVENT                   DMSO
NS                         160
DS                           0
SWH                   5387.931 Hz
FIDRES                0.164427 Hz
AQ                    3.0408704 sec
RG                        1024
DW                      92.800 usec
DE                       6.00 usec
TE                      297.6 K
D1                    1.00000000 sec
TD0                           1

====== CHANNEL f1 ======
NUC1                         1H
P1                       12.00 usec
PL1                      -1.00 dB
SFO1                300.1318534 MHz

F2 - Processing parameters
SI                           32768
SF                  300.1300006 MHz
WDW                             EM
SSB                  0
LB                            0.30 Hz
GB                   0
PC                            1.00
```

^{13}C NMR OF C7




```
Current Data Parameters
NAME                  dec05sisi
EXPNO                         2
PROCNO                        1

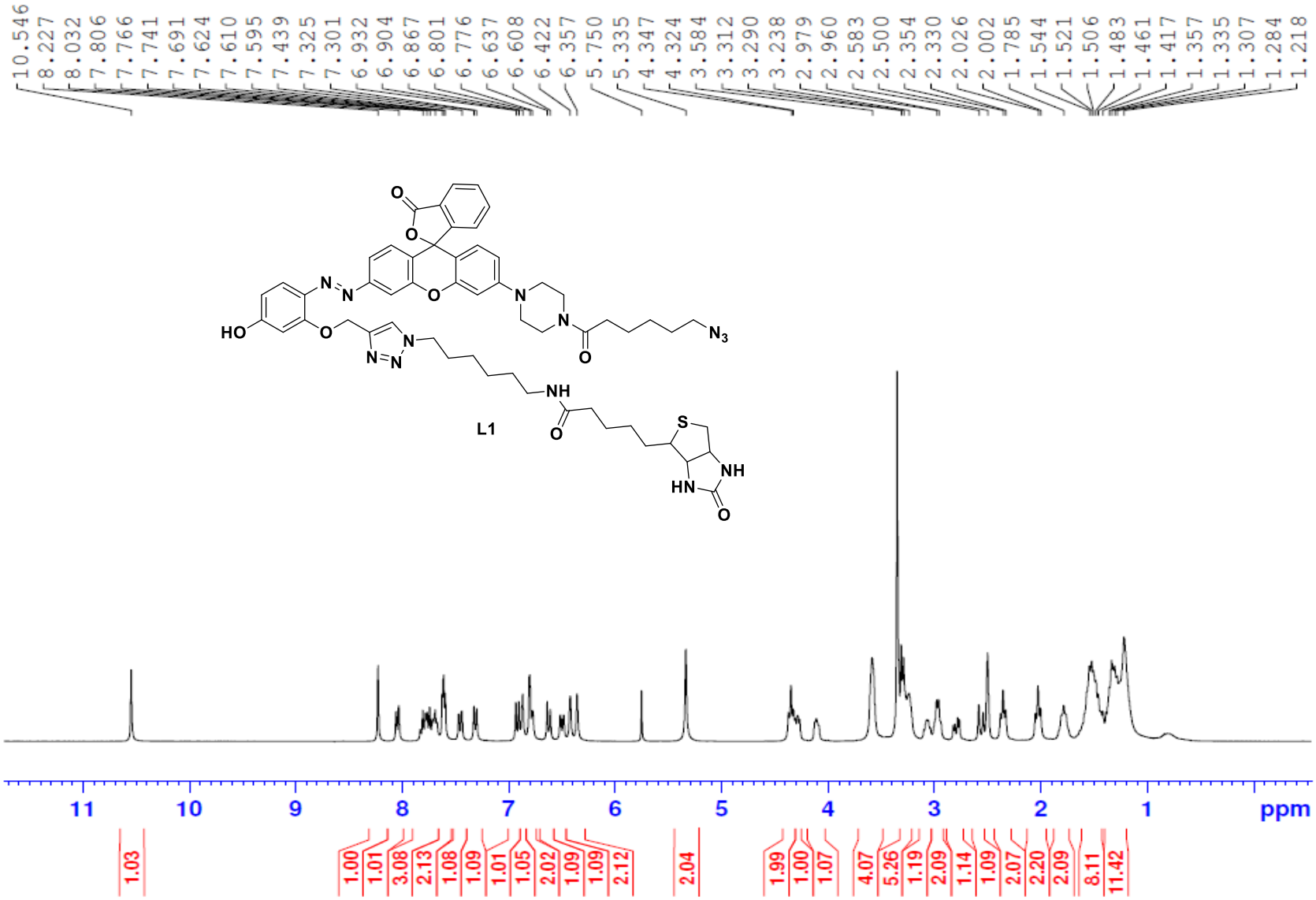
F2 - Acquisition Parameters
Date_                  20161205
Time                      17.53
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                 zgpg30
TD                         32768
SOLVENT                  CDCl3
NS                         27259
DS                             2
SWH                 17985.611 Hz
FIDRES                0.548877 Hz
AQ                    0.9109504 sec
RG                         32768
DW                       27.800 usec
DE                        6.00 usec
TE                       299.7 K
D1                    1.00000000 sec
d11                   0.03000000 sec
DELTA                 0.89999998 sec
TD0                            1

====== CHANNEL f1 ======
NUC1                        13C
P1                        7.80 usec
PL1                       2.00 dB
SFO1                75.4756731 MHz

====== CHANNEL f2 ======
CPDPRG[2                waltz16
NUC2                        1H
PCPD2                    80.00 usec
PL2                      -1.00 dB
PL12                     15.48 dB
PL13                     21.00 dB
SFO2               300.1312005 MHz

F2 - Processing parameters
SI                         32768
SF                  75.4677887 MHz
WDW                           EM
SSB                0
LB                        1.00 Hz
GB                 0
PC                        1.40
```

^{1}H NMR OF P1



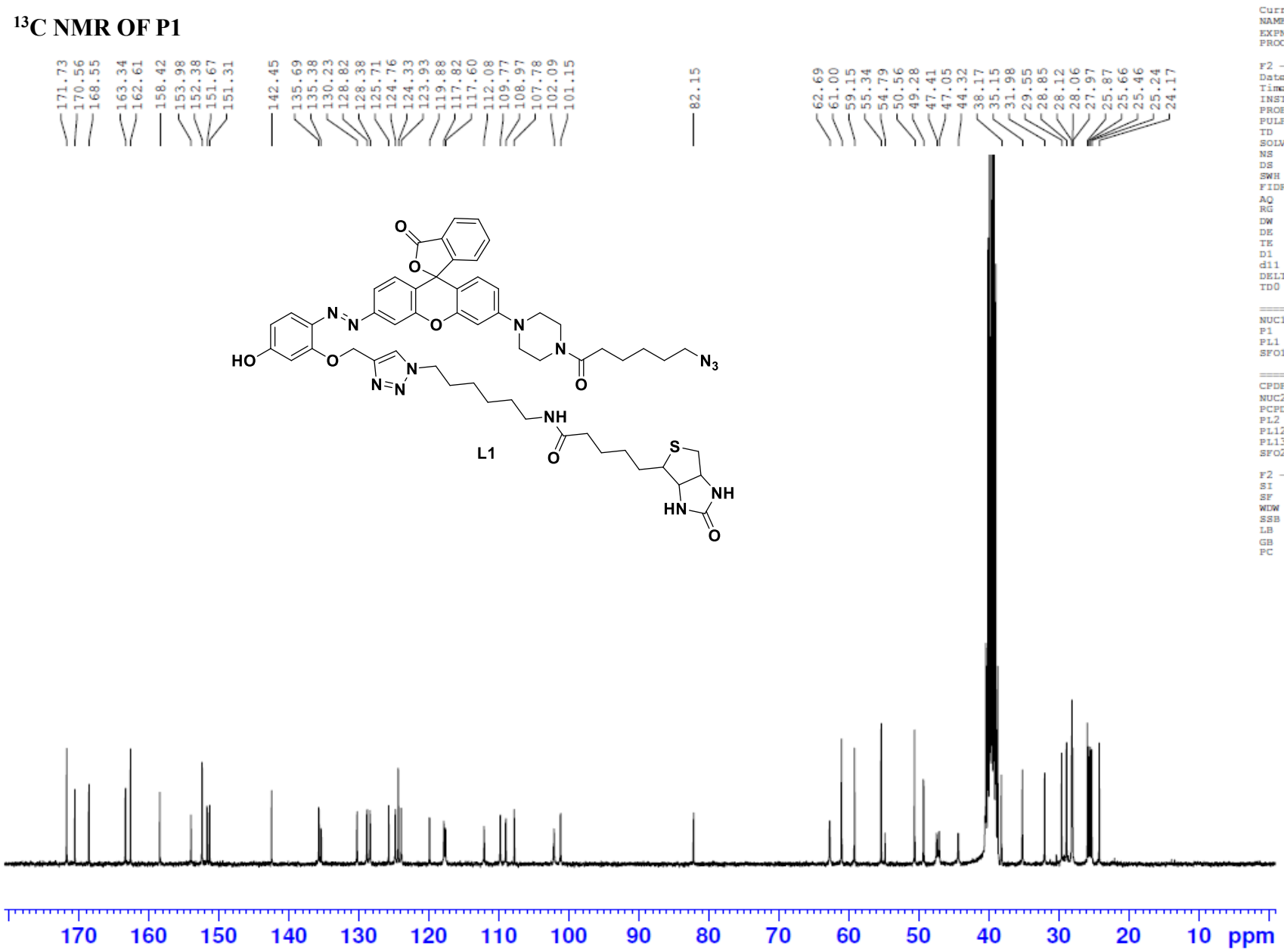

```
Current Data Parameters
NAME                 dec01sisi
EXPNO                        1
PROCNO                       1

F2 - Acquisition Parameters
Date_               20161201
Time                    9.16
INSTRUM                spect
PROBHD    5 mm QNP 1H/13
PULPROG                 zg30
TD                     32768
SOLVENT                 DMSO
NS                        33
DS                         0
SWH                5387.931 Hz
FIDRES            0.164427 Hz
AQ                3.0408704 sec
RG                    287.4
DW                   92.800 usec
DE                    6.00 usec
TE                   298.6 K
D1                1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                      1H
P1                    12.00 usec
PL1                   -1.00 dB
SFO1           300.1318534 MHz

F2 - Processing parameters
SI                     32768
SF             300.1300001 MHz
WDW                       EM
SSB          0
LB                     0.30 Hz
GB           0
PC                     1.00
```

^{13}C NMR OF P1




```
Current Data Parameters
NAME                  dec02sisi
EXPNO                         2
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20161202
Time                      18.56
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                 zqpg30
TD                         32768
SOLVENT                    DMSO
NS                         29283
DS                             2
SWH                   17985.611 Hz
FIDRES                0.548877 Hz
AQ                    0.9109504 sec
RG                         32768
DW                        27.800 usec
DE                         6.00 usec
TE                        298.7 K
D1                    1.00000000 sec
d11                   0.03000000 sec
DELTA                 0.89999998 sec
TD0                            1

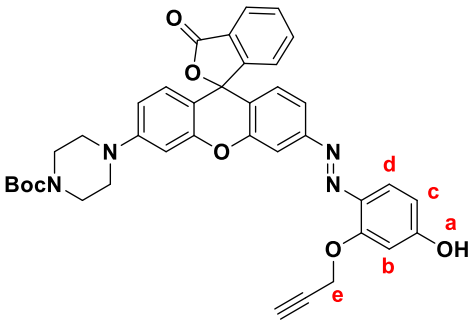
======== CHANNEL f1 ========
NUC1                         13C
P1                         7.80 usec
PL1                        2.00 dB
SFO1                  75.4756731 MHz

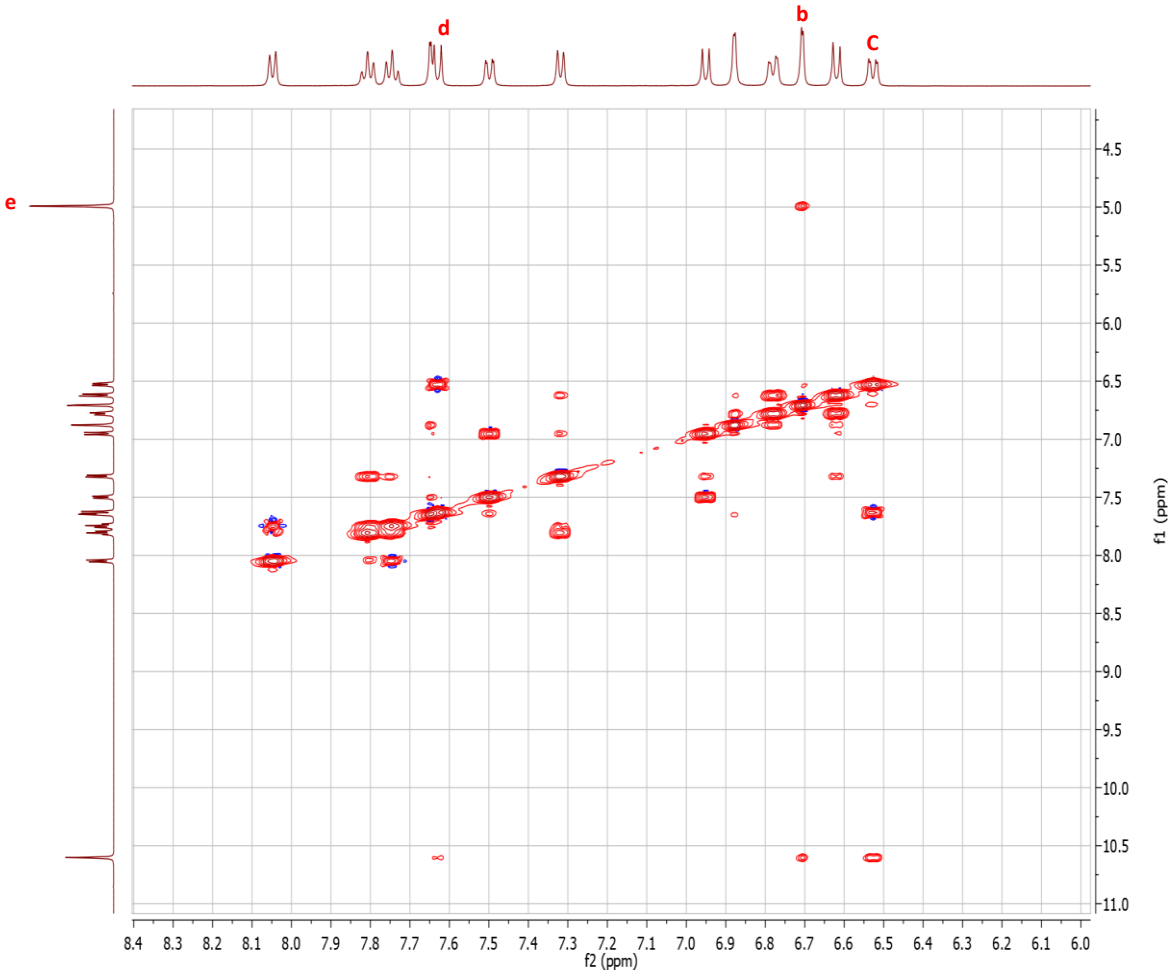
======== CHANNEL f2 ========
CPDPRG[2                waltz16
NUC2                         1H
PCPD2                     80.00 usec
PL2                       -1.00 dB
PL12                      15.48 dB
PL13                      21.00 dB
SFO2                300.1312005 MHz

F2 - Processing parameters
SI                         32768
SF                    75.4677894 MHz
WDW                           EM
SSB                    0
LB                         1.00 Hz
GB                     0
PC                         1.40
```

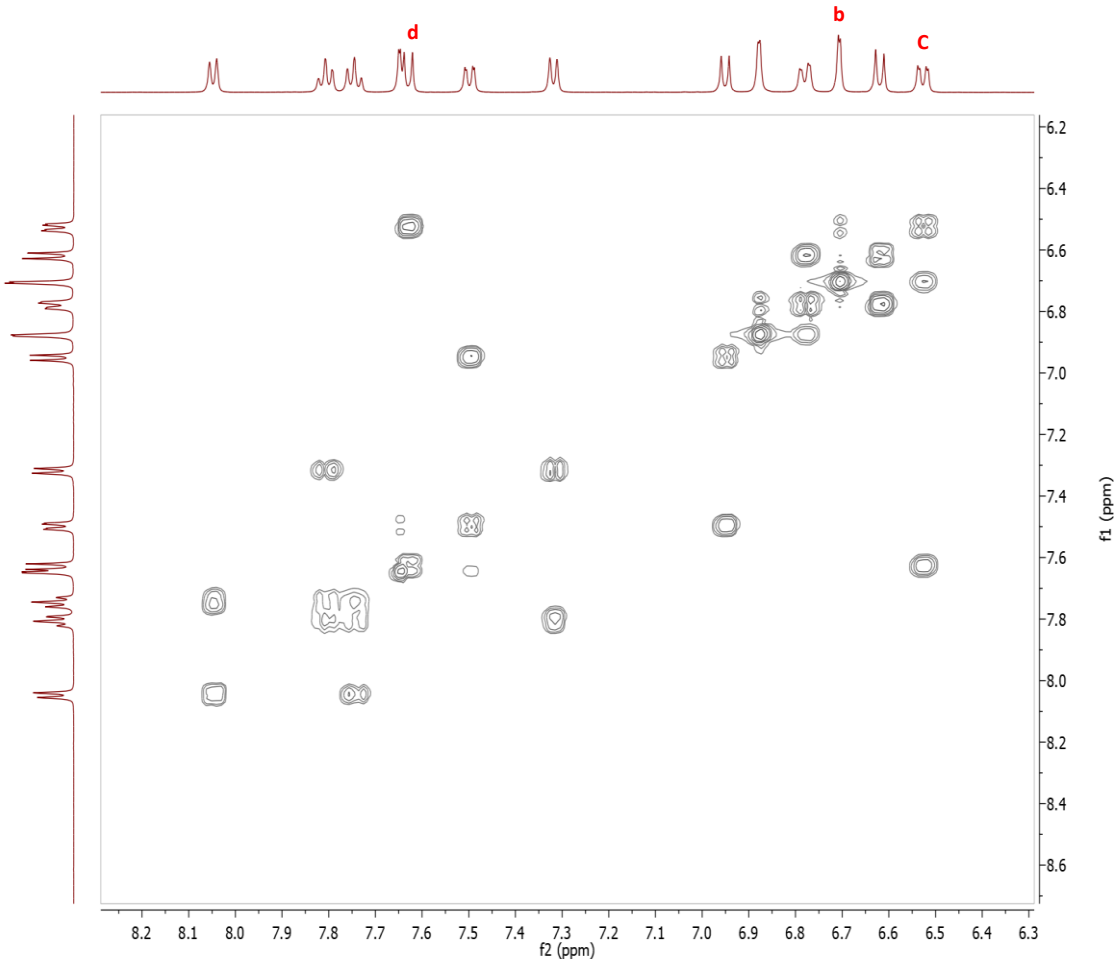
5.3 NOESY & COSY

NOESY OF C6

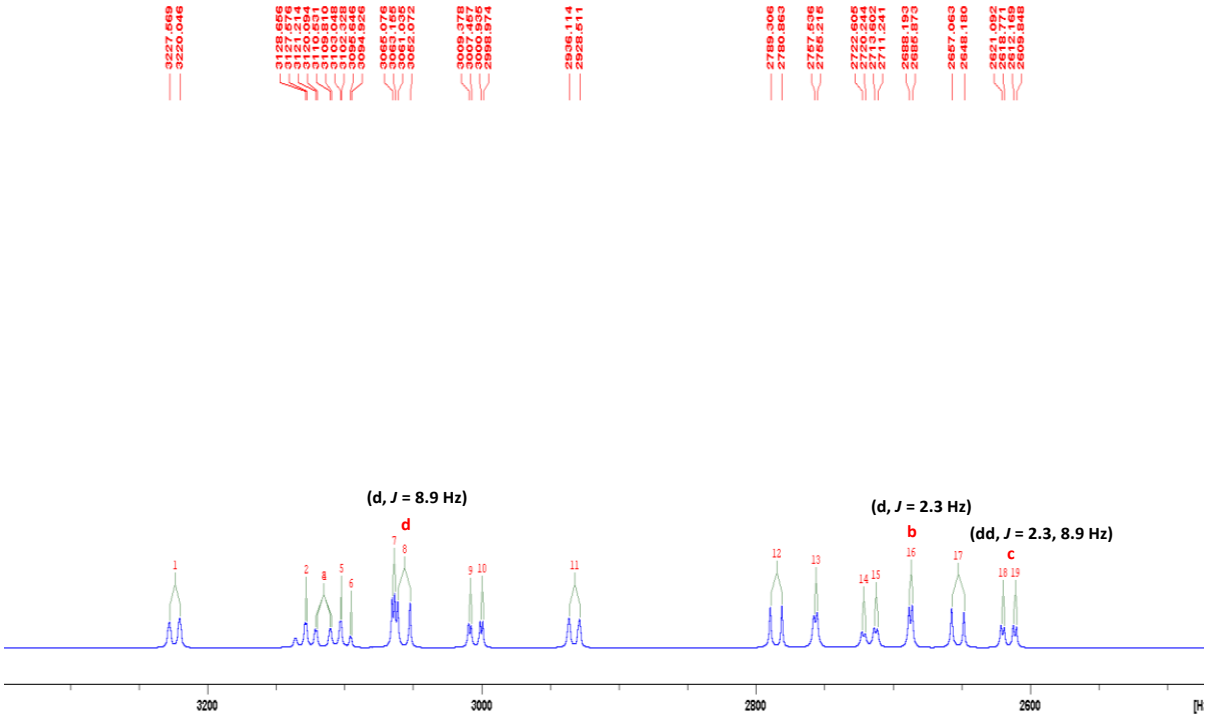




COSY OF C6



J-J Coupling OF C6



6. References

[1] J. B. Grimm and L. D. Lavis, *Org. Lett.*, 2011, **13**, 6354-6357.

[2] J. Ge, C. J. Zhang, L. Li, L. M. Chong, X. Wu, P. Hao, S. K. Sze and S. Q. Yao, *ACS Chem. Biol.*, 2013, **8**, 2577-2585.

[3] Y. Y. Yang, M. Grammel, A. S. Raghavan, G. Charron and H. C. Hang, *Chem. Biol.*, 2010, **17**, 1212-1222.

[4] J. Ge, C. W. Zhang, X. W. Ng, B. Peng, S. Pan, S. Du, D. Wang, L. Li, K. L. Lim, T. Wohland and S. Q. Yao, *Angew. Chem., Int. Ed.*, 2016, **55**, 4933-4937.