Electronic Supplementary Information

Bioisostere-conjugated fluorescent probes for live-cell protein imaging without nonspecific organelle accumulation.

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Abbreviations

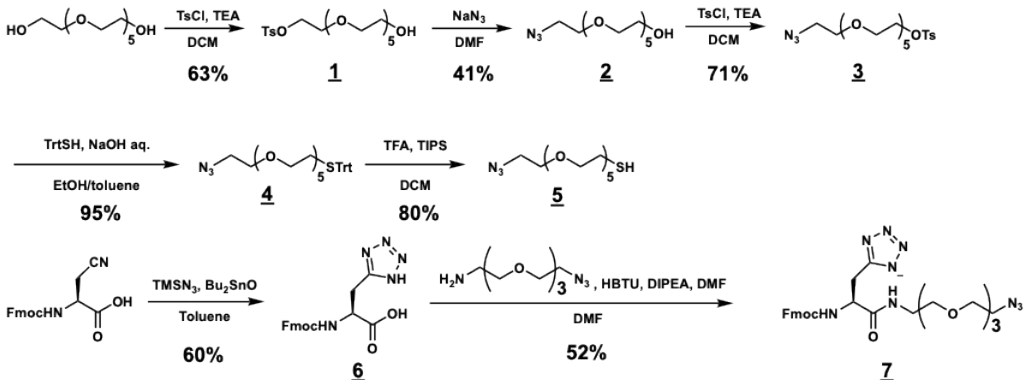
AcOEt, ethyl acetate; Boc, *tert*-butoxycarbonyl; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMEM, Dulbecco's modified Eagle's medium; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; ESI, electrospray ionization; ε, molar extinction coefficient; EtOH, ethanol; FAB, fast atom bombardment; FBS, fetal bovine serum; f.c., final concentration; Φ_{fl}, fluorescence quantum yield; Fmoc, 9-fluorenylmethyloxycarbonyl; GLUT4, glucose transporter type 4; H_2O, water; HBSS, Hanks' Balanced Salt Solution; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometer; KRB, Krebs-Ringer bicarbonate; MeCN, acetonitrile; MeOH, methanol; NaHCO3, sodium hydrogen carbonate; NLS, nuclear localization signal; NMR, nuclear magnetic resonance; Na_2SO_4, sodium sulfate; PBS, phosphate-buffered saline; PS, penicillin-streptomycin; PYP, photoactive yellow protein; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran TLC, thin layer chromatography; TMS, tetramethylsilane, Ts, *p*-toluenesulfonyl

Materials and Instruments

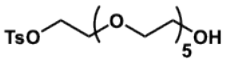
General chemicals were supplied by Tokyo Chemical Industries, Wako Pure Chemical Industries, Sigma-Aldrich Chemical Co., and Kishida Chemical Co., and used without further purification. NMR spectra were recorded on a BRUKER AscendTM 500 instrument at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR or on a JEOL JNM-ECA 600 spectrometer using TMS as an internal standard. FAB-High-resolution mass spectra (HRMS) were recorded using a JEOL JMS-700 instrument. ESI-HRMS was performed using a MicrOTOF II-BC instrument (Bruker). Silica gel chromatography was performed using BW-300 silica (Fuji Silysia Chemical Ltd.) or a Biotage® IsoleraTM One instrument with a pre-packed Biotage® SNAP HC D column or Sfär C18 column. HPLC purification was performed using an Inertsil ODS-3 column (4.6 or 10.0 mm × 250 mm, GL-Science, Inc.) and a TSKgel ODS-80Ts (4.6 or 20.0 mm × 250 mm, TOSOH Co.). Size exclusion chromatography was performed using a SuperdexTM 75 10/300 GL column (GE Healthcare Life Science) connected to an NGC Chromatography System (Bio-Rad). Fluorescence spectra were measured using an F7000 spectrometer (Hitachi) fitted with a photomultiplier at a voltage of 700 V. UV-vis absorption spectra were obtained using a V-650 spectrometer (Jasco). Live fluorescent cell images were acquired using a confocal laser-scanning microscope (Zeiss, LSM-880 or Olympus, Fv10i) or a fluorescence microscope (Keyence, BZ-X710) with a 63× or 60× lens, respectively.

1. Synthetic Procedures

1.1 Linker moiety synthesis



Compound 1

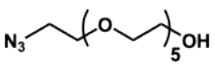


1

Hexaethylene glycol (20.0 g, 70.8 mmol) and TEA (19.8 mL, 142 mmol) were dissolved in DCM (42 mL) and cooled to 0 °C. TsCl (3.38 mg, 17.7 mmol) dissolved in DCM (10 mL) was added dropwise to the reaction mixture, which was then stirred at room temperature for 3 h. The reaction mixture was washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residual material underwent purification through silica gel column chromatography (DCM/MeOH = 95/5 to 90/10 as eluent) to yield compound **1** (4.86 g, 11.2 mmol) with a 63% overall yield.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 4.16 (t, *J* = 5.0 Hz, 2H), 3.72-3.58 (m, 22H), 2.77 (br, 1H), 2.45 (s, 3H). **^{13}C NMR (125 MHz, $CDCl_3$):** δ 144.8, 133.0, 129.8, 128.0, 72.7, 72.6, 70.7, 70.6, 70.6, 70.5, 70.5, 70.5, 70.3, 70.2, 69.3, 68.7 61.7, 61.7, 21.6. **HRMS (ESI+)**: Calcd for $[M+Na]^+$ 459.1659, found 459.1670

Compound 2

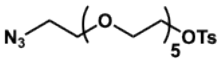


2

NaN_3 (1.95 g, 30.0 mmol), compound **1** (2.65 g, 6.00 mmol), and DMF (6 mL) were stirred at 50 °C overnight. The white precipitate was removed by filtration and the residue was washed with AcOEt. The filtrate was concentrated *in vacuo*, then the resultant was purified by silica gel column chromatography (Hexane/AcOEt = 50/50 to 20/80 as eluent) to yield compound **2** (740 mg, 2.44 mmol) with a 41% overall yield.

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.73-3.60 (m, 22H), 3.39 (t, *J* = 5.0 Hz, 2H), 2.20 (br, 1H), **^{13}C NMR (125 MHz, $CDCl_3$):** δ 72.6, 70.7, 70.6, 70.6, 70.6, 70.5, 70.3, 70.0, 61.7, 50.7, 36.5, 31.4. **HRMS (ESI+):** Calcd for $[M+Na]^+$ 330.1636, found 330.1652

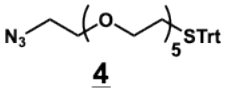
Compound 3



3

TsCl (4.51 g, 23.7 mmol) in DCM (72 mL) was added dropwise to a stirred solution of compound **2** (2.43 g, 7.89 mmol) and TEA (3.30 mL, 23.7 mmol) in DCM (40 mL) at room temperature. Subsequently, the solution was stirred overnight at room temperature. The reaction mixture was washed with water and brine. The solution was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (DCM/MeOH = 99/1 to 90/10 as eluent) to obtain compound **3** (2.68 g, 5.80 mmol) with a 73% overall yield.
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 4.16 (t, *J* = 5.0 Hz, 2H), 3.70-3.38 (m, 22H), 2.45 (s, 3H). **^{13}C NMR (125 MHz, $CDCl_3$):** δ 114.8, 133.0, 129.8, 128.0, 70.8, 70.7, 70.7, 70.6, 70.6, 70.6, 70.5, 70.0, 69.3, 68.7. 21.5. **HRMS (ESI+):** Calcd for $[M+Na]^+$ 484.1724, found 484.1705

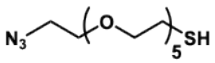
Compound 4



4

NaOH (391 mg, 9.95 mmol) in H_2O (4.4 mL) was added to a solution of triphenylmethanethiol (2.19 g, 7.94 mmol) in ethanol/toluene (1/1) (30 mL). After the addition, compound **3** (3.00 g, 6.50 mmol) in ethanol/toluene (1/1) (30 mL) was added to the solution at room temperature, then the solution was stirred at room temperature for 5 h. The reaction mixture was then washed with sat. $NaHCO_3$ aq. and brine. The solution was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography on silica gel (Hexane/ethyl acetate = 10/90 to 30/70 as eluent) to obtain compound **4** (3.49 g, 6.16 mmol) with a 95% overall yield.
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.41 (m, 6H), 7.27 (m, 6H), 7.20 (m, 3H), 3.65-3.44 (m, 24H), 3.38 (t, *J* = 5.0 Hz, 2H), 3.30 (t, *J* = 7.0 Hz, 2H). **^{13}C NMR (125 MHz, $CDCl_3$):** δ 44.8, 129.6, 127.9, 126.7, 70.7, 70.7, 70.6, 70.6, 70.6, 70.6, 70.5, 70.2, 70.0, 69.6, 66.6, 50.7, 31.6. **HRMS (ESI+):** Calcd for $[M+Na]^+$ 588.2503, found 588.2489

Compound 5

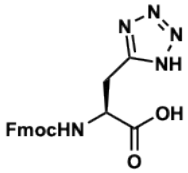


5

TFA (18 mL) was added dropwise to a stirred solution of compound **4** (1.05 g, 1.77 mmol) and triethylsilane (246 mg, 2.21 mmol) in DCM (110 mL) at room temperature and the solution was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (DCM to DCM/MeOH = 90/10 as eluent) to obtain compound **5** (455 mg, 1.41 mmol) with an 80% overall yield.

^{1}H NMR (500 MHz, DMSO-d_6): δ 3.61-3.49 (m, 20H), 3.40 (t, J = 5.0 Hz, 2H), 2.64-2.59 (m, 2H), 2.28 (t, J = 8.0 Hz, 1H). **^{13}C NMR (125 MHz, DMSO-d_6):** δ 72.6, 70.3, 70.3, 70.2, 70.2, 69.9, 69.7, 50.5, 23.9. **HRMS (FAB+):** Calcd for $[M]^+$367.2380, found 367.2390.

Synthesis of compound 6

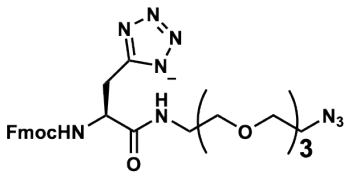


6

A solution of Fmoc-Ala(3-CN)-OH (336 mg, 1.00 mmol), $TMSN_3$ (460 mg, 4.00 mmol) and Bu_2SnO (224 mg, 0.900 mmol) in toluene (5 mL) was stirred at 100 °C for 2 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH = 10/1 to MeOH as eluent) to obtain compound **6** (226 mg, 0.596 mmol) with a 60% overall yield.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.88 (d, J = 7.5 Hz, 2H), 7.83 (d, J = 8.5 Hz, 1H), 7.67-7.30 (m, 6H), 4.49 (ddd, J= 15.0, 8.5, 7.5 Hz, 1H), 4.27-4.19 (m, 3H), 3.40 (dd, 2 J = 15.0, 6.0 Hz), 3.27 (dd, 2 J = 15.0, 6.0 Hz). **^{13}C NMR (125 MHz, DMSO-d_6)** δ 172.5, 156.3, 154.1, 144.2, 141.2, 128.1, 127.6, 125.7, 125.6, 120.6, 66.2, 52.7, 47.0, 25.9. **HRMS (FAB-):** Calcd for $[M]^-$ 378.1208, found 378.1207.

Synthesis of compound 7



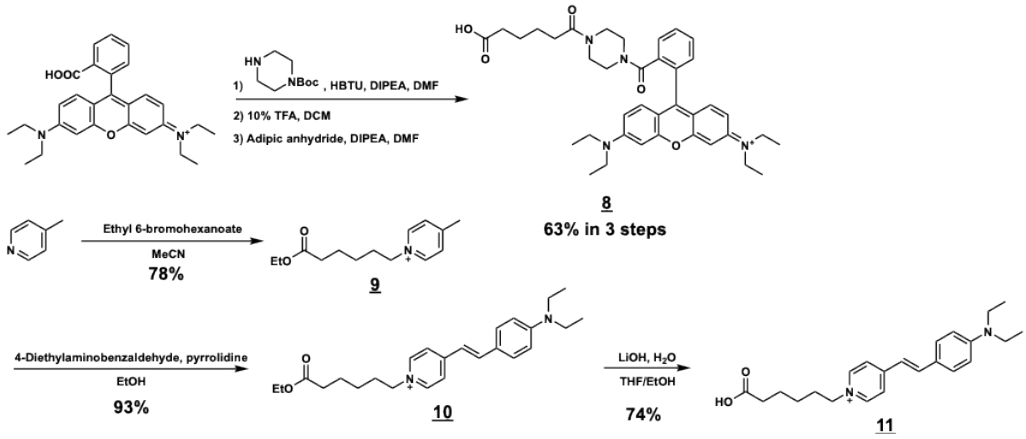
7

DIPEA (306 mg, 2.37 mmol) and compound **6** (300 mg, 0.790 mmol) were dissolved in DMF

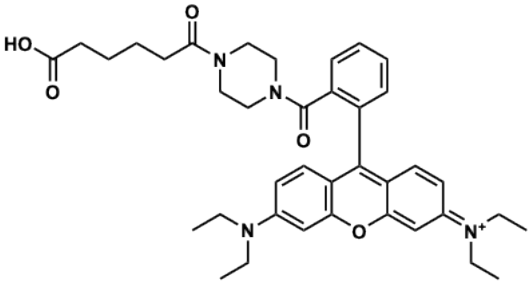
(7.5 mL). HBTU (299 mg, 0.790 mmol) was added to the resulting solution and was stirred at 0 °C for 45 min. 11-Azido-3, 6, 9-trioxaundecan-1-amine (205 mg, 0.940 mmol) was added to the reaction mixture, warmed to room temperature, and stirred overnight. The reaction solution was concentrated *in vacuo*, then the residue was purified by column chromatography (DCM/MeOH = 99/1 to 1/1 as eluent) to yield the title compound (239 mg, 0.410 mmol) as a colorless oil with a 52% overall yield.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.02-7.31 (m, 9H) 4.49-4.46 (m, 1H) 4.29-4.19 (m, 3H), 4.49 (ddd, *J* = 15.0, 8.5, 7.5 Hz, 1H), 4.27-4.19 (m, 3H), 3.58-3.14 (m, 18H). **HRMS (ESI-):** Calcd for $[M-H]^-$ 578.2481, found 578.2457

1.2 Synthesis of cationic dye scaffolds (RB and SP)



Synthesis of compound 8

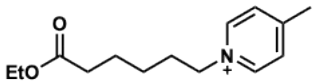


8

Rhodamine B (479 mg, 1.00 mmol), *tert*-butyl 1-piperazinecarboxylate (668 mg, 1.89 mmol), and DIPEA (194 mg, 1.50 mmol) were dissolved in DMF (6.5 mL). COMU (514 mg, 1.20 mmol) was added to the resulting solution at 0 °C and stirred at 0 °C for 45 min. The reaction mixture was heated to room temperature and stirred overnight. The reaction solution was concentrated *in vacuo*, and then 10% TFA in DCM was added to the residue and stirred at room temperature overnight. The reaction solution was concentrated *in vacuo*, and Adipic Anhydride (128 mg, 1.00 mmol) and DIPEA (258 mg, 2.00 mmol) were added to the residue and stirred at 40 °C for 6 h. The reaction solution was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (DCM/MeOH = 99/1 to 90/10 as eluent) to yield compound **8** (405 mg, 0.63 mmol) with a 63% overall yield.

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.80-7.74 (m, 3H), 7.59-7.57 (m, 1H), 7.34 (d, J = 9.6 Hz, 2H), 6.96 (d, J = 3.0 Hz, 2H), 3.78 (q, J = 3.0 Hz, 8H), 3.60-3.31 (m, 8H), 2.33-2.29 (m, 3H), 1.59-1.57 (m, 4H), 1.33 (t, J = 7.2 Hz, 12H). **^{13}C NMR (125 MHz, MeOD-d_4)** 177.2, 174.0, 169.7, 159.4, 157.3, 157.1, 136.6, 133.2, 132.3, 131.8, 131.3, 129.0, 114.5, 114.9, 97.4, 46.9, 43.2, 42.8, 34.6, 33.6, 25.8, 25.6, 12.8. **HRMS (FAB+):** Calcd for $[M]^+$ 639.3541, found 639.3545.

Synthesis of compound 9

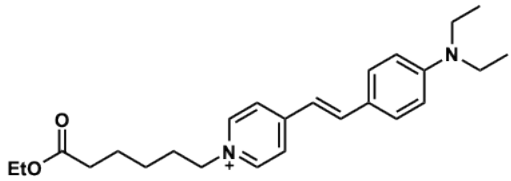


9

4-methylpyridine (2.10 g, 21.5 mmol) and ethyl 6-bromohexanoate (4.60 mL, 25.8 mmol) were dissolved in CH_3CN (40 mL) and the mixture was then heated up to 95 °C under reflux for 18 h. The reaction solution was concentrated *in vacuo* and cold ether/hexane (1/1). After removing the supernatant by decantation, the residue was concentrated *in vacuo* to obtain compound **9** (6.5 g, 27.5 mmol) with a 78% overall yield.

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.37 (d, *J* = 6.5 Hz, 2H), 7.90 (d, *J* = 6.5 Hz, 2H), 4.94 (t, *J* = 7.5 Hz, 2H), 4.10 (q, *J* = 6.5 Hz, 2H), 2.68 (s, 3H), 2.30 (t, *J* = 6.5 Hz, 2H), 2.07 (dd, *J* = 6.5 Hz, 2H), 1.67 (dd, *J* =6.5 Hz, 2H, d), 1.45-1.42 (m, 2H), 1.24 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR (125 MHz, $CDCl_3$):** δ 173.4, 158.9, 144.3, 128.9, 60.8, 60.4, 33.8, 31.5, 25.4, 24.1, 22.3, 14.2. **HRMS (FAB+):** Calcd for $[M]^+$ 236.1645, found 236.1659.

Synthesis of compound 10

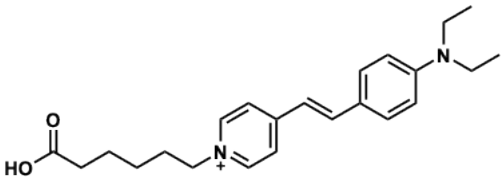


10

4-Diethylaminobenzaldehyde (200 mg, 1.13 mmol) and 1-(6-ethoxy-6-oxohexyl)-4-methylpyridin-1-ium (214 mg, 0.910 mmol) were dissolved in EtOH (7 mL) in a microwave reaction vessel and pyrrolidine (55.8 µL, 0.910 mmol) was added to the solution. The mixture was heated to 120 °C under microwave irradiation for 30 min. The solution was concentrated *in vacuo*, then the residue was purified by flash column chromatography on silica gel (DCM/MeOH = 97/3 as eluent) to yield compound **10** (336 mg, 0.850 mmol) with a 93% overall yield.

^{1}H NMR (500 MHz; $CDCl_3$): δ 8.94 (d, *J*= 7.0 Hz, 2H), 7.80 (d, *J*= 7.0 Hz, 2H), 7.59 (d, *J*= 16 Hz, 1H), 7.51 (d, *J*= 9 Hz, 2H), 6.83 (d, *J*= 16 Hz, 1H, 6.67 (d, *J*= 9 Hz, 2H), 4.69 (t, 2H), 4.10 (q, *2*H), 3.44 (t, 4H), 2.31 (t, 2H), 2.02 (tt, 2H), 1.67 (m, 2H), 1.44 (m, 2H), 1.23 (m, 9H) ; **^{13}C NMR (125 MHz, $CDCl_3$):** δ 173.4, 154.3, 150.3, 143.3, 143.2, 131.1, 122.4, 121.5, 115.6, 111.5, 60.4, 59.8, 44.7, 33.7, 31.3, 25.4, 24.1, 14.2, 12.5. **HRMS (FAB+):** Calcd for $[M]^+$395.2693, found 395.2698.

Synthesis of compound 11

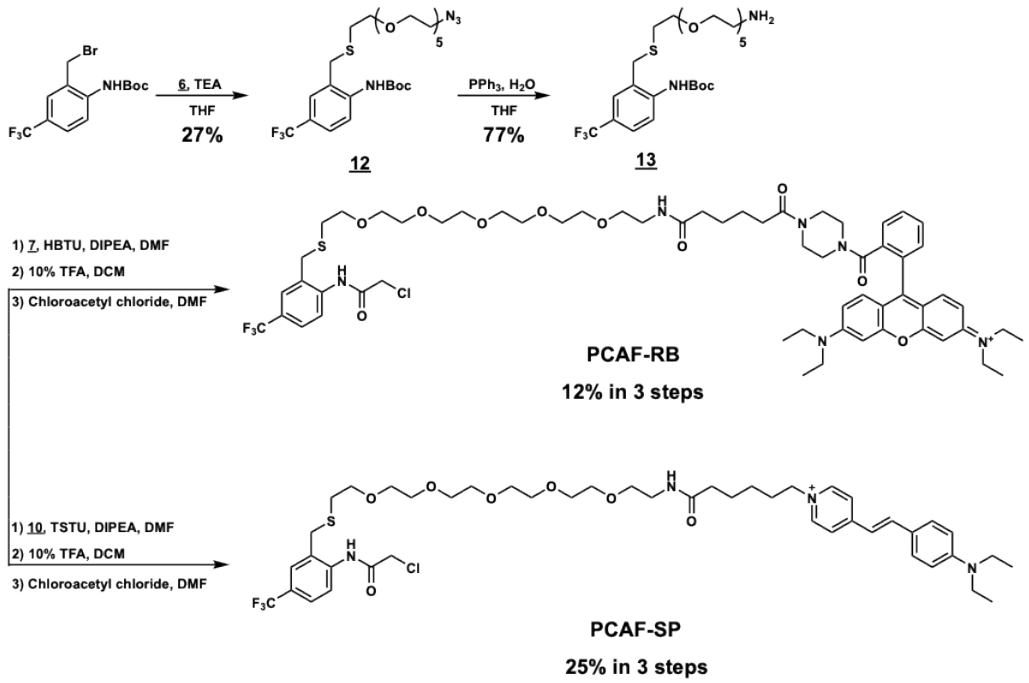


11

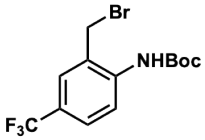
THF (4 mL) and EtOH (2 mL) were added to compound **10** (174 mg, 0.477 mmol), mixed with LiOH (100 mg, 2.38 mmol) in water (2 mL), and stirred for 24 h at room temperature. The solution was concentrated *in vacuo*, then the residue was purified by flash column chromatography on silica gel (DCM/MeOH = 80/20 as eluent) to yield compound **11** (130 mg, 0.354 mmol) with a 74% overall yield.

^{1}H NMR (500 MHz; MeOD-d_4): δ 8.56 (d, J = 7.0 Hz, 2H), 7.94 (d, J = 7.0 Hz, 2H), 7.81 (d, J = 16 Hz, 1H), 7.58 (d, J = 9 Hz, 2H,), 7.05 (d, J = 16 Hz, 1H), 6.75 (d, J = 9 Hz, 2H), 4.41 (t, 2H), 3.48 (q, 4H), 2.18 (t, 2H), 1.97 (tt, 2H), 1.66 (tt, 2H), 1.67 (m, 2H), 1.39 (tt, 2H), 1.20 (t, 6H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 182.3, 156.4, 151.6, 144.5, 144.3, 132.0, 123.6, 123.5, 117.2, 112.7, 60.9, 45.5, 38.6, 31.9, 26.9, 26.7, 12.9. **HRMS (FAB+):** Calcd for $[M]^+$367.2380, found 367.2390.

1.3 Synthesis of **PCAF-RB** and **PCAF-SP**

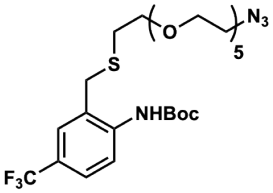


Synthesis of *tert*-butyl (2-(bromomethyl)-4-(trifluoromethyl)phenyl)carbamate



This compound was synthesized following a reported procedure.[S1]

Compound 12



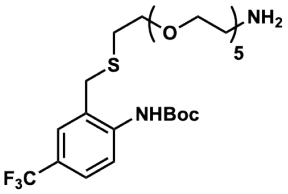
12

tert-Butyl (2-(bromomethyl)-4-(trifluoromethyl)phenyl)carbamate (668 mg, 1.89 mmol), compound **5** (610 mg, 1.89 mmol), and TEA (0.520 mL, 3.77 mmol) were dissolved in THF (60 mL) at room temperature overnight. The reaction solution was concentrated *in vacuo*, then the residue was purified by flash column chromatography on silica gel (Hexane/ethyl acetate = 70/30 to 30/70 as eluent) to yield compound **12** (303 mg, 507 μmol) with a 27% overall yield.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (d, *J* = 8.5 Hz, 1H), 7.64 (s, 1H), 7.51 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.44 (d, *J* = 2.0 Hz, 1H), 3.89 (s ,2H), 3.71 (t, *J* = 6.0 Hz, 2H), 3.67-3.62 (m, 18H), 3.38 (t, *J* = 5.0 Hz, 2H), 2.58 (t, *J* = 6.0 Hz, 2H), 1.54 (s, 9H). **^{13}C NMR (125 MHz, $CDCl_3$):** δ 152.8,

140.5, 127.6 (q, $^{3}J_{C\text{-}F}$ = 3.8 Hz), 126.3, 125.4 (q, $^{3}J_{C\text{-}F}$ = 3.8 Hz), 124. 8 (q, $^{2}J_{C\text{-}F}$ = 32.5 Hz), 124.1 (q, $^{1}J_{C\text{-}F}$ = 268.8 Hz), 121.3, 81.1, 71.9, 70.7, 70.6, 70.6, 70.6, 70.6, 70.4, 70.0, 50.7, 33.6, 30.4, 28.3. **HRMS (FAB+):** Calcd for $[M+Na]^{+}$ 619.2384, found 619.2394.

Compound 13

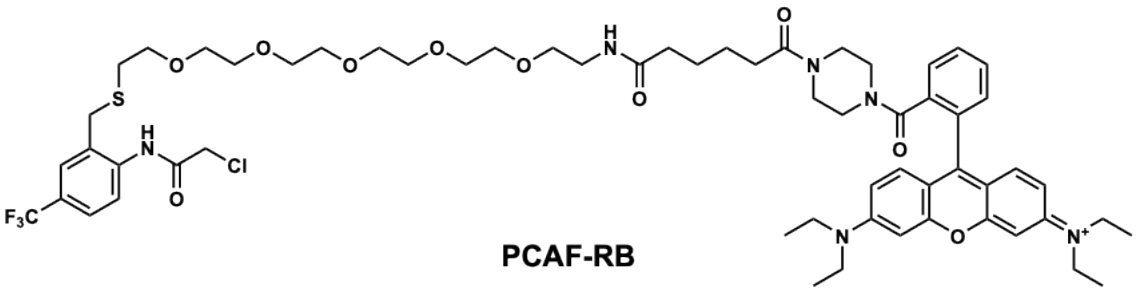


13

Triphenylphosphine (52.8 mg, 201 μmol) and compound **12** (99.4 mg, 168 μmol) were dissolved in THF (5 mL) and the mixture was then heated up to 60 °C for 2 h. After stirring, the solution was cooled at room temperature and water was added to it (500 μL). The resulting solution was stirred at room temperature for 5 h. The reaction solution was concentrated *in vacuo*, then the residue was purified by silica gel column chromatography (DCM/MeOH = 10/1 to MeOH as eluent) to yield compound **13** (73.3 mg, 128 μmol) with a 77% overall yield.

^{1}H NMR (500 MHz; $CDCl_3$): δ 8.07 (d, *J* = 8.5 Hz, 1H), 7.63 (br, 1H), 7.51 (dd, *J* = 8.5, 2.0 Hz, 1H, d), 7.44 (d, *J* = 2.0 Hz, 1H), 3.90 (s, 2H), 3.71 (t, *J* = 6.0 Hz, 2H), 3.67-3.61 (m, 16H), 3.50 (t, *J* = 5.0 Hz, 2H) , 2.85 (t, *J* = 5.0 Hz, 2H), 2.58 (t, *J* = 6.0 Hz, 2H), 1.54 (s, 9H). **^{13}C NMR (125 MHz, $CDCl_3$):** δ 152.8, 140.5, 127.6 (q, $^{3}J_{C\text{-}F}$ = 3.8 Hz), 126.4, 125.3 (q, $^{3}J_{C\text{-}F}$ = 3.8 Hz), 124.5 (q, $^{2}J_{C\text{-}F}$ = 32.5 Hz), 124.1 (q, $^{3}J_{C\text{-}F}$ = 270.0 Hz), 121.4, 81.1, 72.7, 71.7, 70.6, 70.5, 70.5, 70.3, 70.3, 41.6, 33.6, 30.4, 28.3. **HRMS (ESI+):** Calcd for $[M+H]^{+}$ 571.2659, found 571.2652.

Synthesis of PCAF-RB



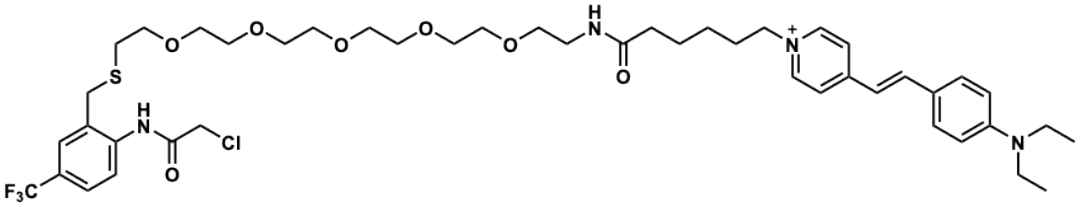
PCAF-RB

DIPEA (6.0 mg, 46 μmol) and compound **8** (10 mg, 16 μmol) were dissolved in DMF (2 mL). HBTU (9.0 mg, 23 μmol) was added to the resulting solution at 0 °C and stirred at 0 °C for 45 min. Compound **13** was added to the reaction mixture (15 mg, 23 μmol) that was warmed to room temperature and stirred overnight. The reaction solution was concentrated *in vacuo*, then 10% TFA in DCM was added to the residue at 0 °C and stirred for 2 h at 0 °C. The reaction solution was concentrated *in vacuo*, and the residue was dissolved in DMF. Chloroacetyl chloride (23 mg,

200 μmol) was added to the reaction solution at 0 °C and stirred for 1 h at 0 °C. The reaction solution was concentrated *in vacuo*, then the residue was purified by reversed-phase HPLC using ODS-3 column and eluted with H_2O/CH_3CN containing 0.1% TFA to yield the title compound (3.1 mg, 2.7 μmol) with a 12% overall yield.

^{1}H NMR (600 MHz; MeOD-d_4): δ 7.89 (d, J = 7.8 Hz, 1H), 7.78-7.71 (m, 4H), 7.06 (d, J = 7.8 Hz, 1H), 7.52 (br, 1H), 7.28 (d, J = 9.0 Hz, 2H), 7.07 (dd, J = 9.0, 1.8 Hz, 1H), 6.96 (d, J = 1.8 Hz, 2H), 4.34 (s, 2H), 3.97 (s, 2H), 3.68 (q, J = 7.2 Hz, 8H), 3.66-3.38 (m, 22H), 3.21 (q, J = 7.2 Hz, 2H), 2.56 (t, J = 6.0 Hz, 2H), 2.35 (t, J = 7.2 Hz, 2H), 2.19 (br, 1H), 1.59-1.27 (m, 4H), 1.32-1.29 (m, 12H). **^{13}C NMR (125 MHz, MeOD-d_4):** 175.9, 167.8, 159.3, 157.3, 157.1, 140.3, 136.6, 133.6, 133.3, 131.8, 131.4, 128.9 (q, $^2J_{C\text{-}F}$ = 31.0 Hz), 128.8 (q, $^3J_{C\text{-}F}$ = 3.5 Hz), 126.4, 126.4, 125.9 (q, $^3J_{C\text{-}F}$ = 3.5 Hz), 126.4, 124.4, 123.5 (q, $^1J_{C\text{-}F}$ = 270 Hz), 115.5, 107.5, 97.4, 72.7, 71.6, 71.3, 71.3, 70.6, 48.0, 47.0, 40.4, 36.6, 33.5, 31.8, 25.8, 12.9, 9.3. **HRMS (ESI+):** Calcd for $[M+H]^+$ 1167.5214, found 1167.5267.

Synthesis of PCAF-SP



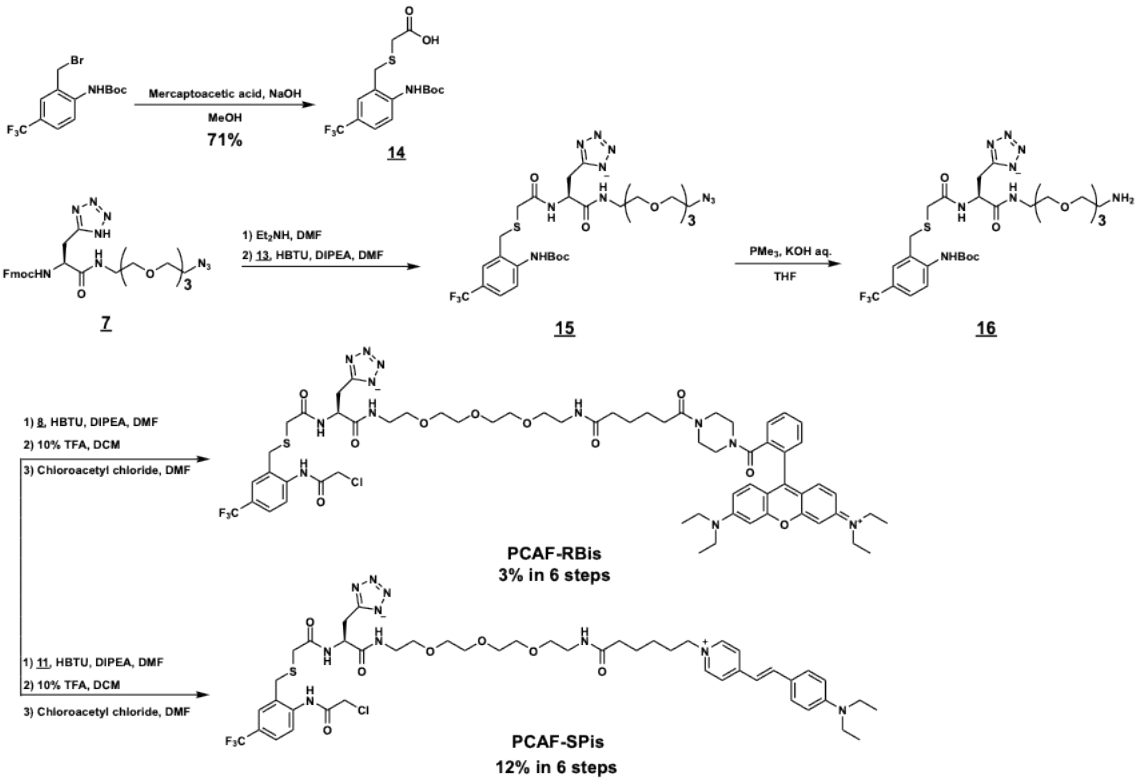
PCAF-SP

TSTU (5.7 mg, 19 μmol), compound **13** (57 μmol), compound **11** (7.0 mg, 1.9 μmol), and DIPEA (9.9 μL, 57 μmol) were dissolved in DMF (10 mL) and stirred for 12 h at 0 °C under a N_2 atmosphere. The reaction solution was concentrated *in vacuo* and the residue was dissolved in DCM (5 mL) and TFA (1 mL) at room temperature and stirred for 1 h at room temperature. The reaction solution was concentrated *in vacuo*, and the residue was dissolved in DCM (5 mL). Chloroacetyl chloride (1.9 μL, 23 μmol) was added to the reaction solution at room temperature and stirred for 1 h at room temperature. The reaction solution was concentrated *in vacuo*, and the residue was purified by reverse-phase HPLC using an ODS-3 column and eluted with H_2O/CH_3CN containing 0.1% TFA to yield **PCAF-SP** (4.51 mg) with a 25% overall yield.

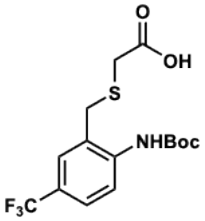
^{1}H NMR (500 MHz; $CDCl_3$): δ8.55 (d, J = 7.0 Hz, 1H), 7.95 (d, J = 7.0 Hz, 1H, H_z), 7.88 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 16.0 Hz, 1H,), 7.71 (s, 1H), 7.60-7.58 (m, 3H), 7.06 (d, J = 16.0 Hz, 1H, H_{aa}), 6.79 (d, J = 8.5 Hz, 2H, H_{ad}),4.41 (t, J = 7.5 Hz, 2H), 4.34 (s, 2H), 3.96 (s, 2H),3.65-3.33 (m, 24H) , 2.59 (t, J = 6.0 Hz, 2H), 2.22 (t, J = 7.0 Hz, 2H), 1.97 (tt, 2H), 1.67 (tt, 2H), 1.36 (tt, 2H), 1.20 (t, J = 7.0 Hz, 6H) ; **^{13}C NMR (125 MHz, MeOD-d_4):** 174.3, 166.4, 155.0, 143.1, 142.9, 138.8, 132.1, 130.6, 127.3, 127. 3, 128.5 (q, $^3J_{C\text{-}F}$ = 3.8 Hz),125.0, 124.4, 123.3 (q, $^1J_{C\text{-}F}$ = 270 Hz), 71.2, 70.2, 70.2, 70.2, 70.1, 70.1, 70.1, 70.1, 70.1, 69.9, 69.8, 69.1, 59.5, 44.5, 42.5, 38.9, 34.9, 32.1, 30.3,

25.0, 24.6, 11.4. **HRMS (ESI+)**: Calcd for $[M]^+$ 895.4053, found 895.4093.

1.4 Synthesis of **PCAF-RBis** and **PCAF-SPis**



Synthesis of compound 14



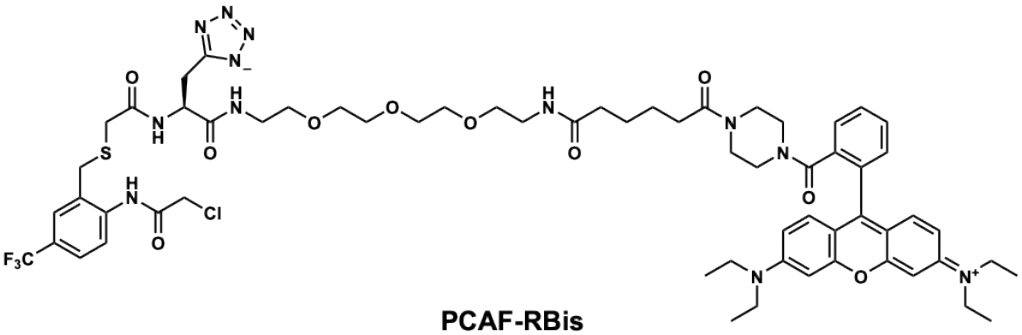
14

tert-Butyl (2-(bromomethyl)-4-(trifluoromethyl)phenyl)carbamate (708 mg, 2.00 mmol) was dissolved in MeOH (4.0 mL) and added to a solution of mercaptoacetic acid (404 mg, 4.40 mmol) in MeOH (10.0 mL). The reaction mixture was stirred at room temperature. Then, a solution of NaOH (160 mg, 4.00 mmol) in MeOH (3.0 mL) was slowly added, and the resulting mixture was stirred at room temperature until benzyl bromide was absent (checked by TLC). The resulting solution was diluted with 2 M NaOH and extracted with DCM/MeOH. The combined organic fractions were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified using column chromatography (DCM/MeOH = 95/5 to 50/50 as eluent) to yield the title compound (518 mg, 1.42 mmol) as a colorless oil with a 71% overall yield.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.90 (br, 1H), 7.51 (d, J = 9.0 Hz, 1H), 7.44 (s, 1H), 3.88 (br,

2H), 3.17 (s, 2H), 1.53 (s, 9H) **^{13}C NMR (150 MHz, $CDCl_3$)**: δ 175.7, 153.8, 140.1, 127.5, 125.6, 125.3, 123.9 (q, $^1J_{C\text{-}F}$ = 270 Hz), 122.1, 82.3, 33.2, 32.1, 28.2. **HRMS (ESI-):** Calcd for $[M\text{-}H]^-$ 364.0836, found 364.0836.

Synthesis of PCAF-RBis

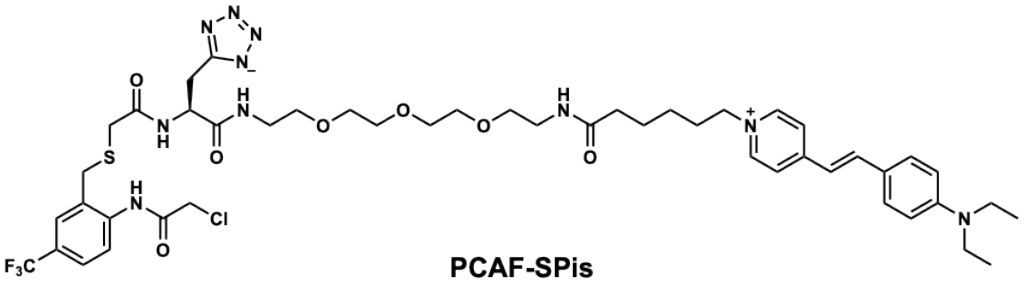


PCAF-RBis

We added 10% Diethyl amine in DMF (5 mL) to compound **7** (321 mg, 0.550 mmol) and the mixture was stirred at room temperature overnight. The reaction solution was concentrated *in vacuo*, and the crude product was used for the next step. To a solution of compound **14** (303 mg, 0.830 mmol) we added DIPEA (214 mg, 1.66 mmol) and HBTU (472 mg, 1.24 mmol) in DMF (5 mL) at 0 °C and stirred at 0 °C for 45 min. The crude product was heated to room temperature, added to the reaction mixture, and stirred overnight. The reaction solution was concentrated *in vacuo*, then the residue was purified by column chromatography (H_2O/MeCN containing 0.1% formic acid) to yield the crude product of compound **15** (118 mg) as a colorless oil. A part of the crude product (43 mg) was dissolved in THF/water (2:1, v/v). 1M trimethylphosphine in THF (240 μL, 240 μmol) and KOH (13 mg, 240 μmol) were added to the mixture at 0 °C. The reaction solution was stirred at 0 °C overnight. The reaction solution was concentrated *in vacuo*, and the crude product was separated by silica gel column chromatography (DCM/MeOH = 50/50 to 10/90 as eluent)) for the next coupling reaction. Compound **8** (10 mg, 16 μmol) and DIPEA (6 mg, 46 μmol) were dissolved in DMF (2 mL). HBTU (9 mg, 23 μmol) was added at 0 °C and stirred at 0 °C for 45 min. The reaction mixture was added to a crude Staudinger reaction mixture (crude amino derivative), warmed to room temperature, and stirred overnight. The reaction solution was concentrated *in vacuo*, and the crude product was prepared by reverse phase silica gel column chromatography (H_2O/MeCN containing 0.1% formic acid) for the next step. We added 10% TFA in DCM to the residue at 0 °C and stirred the mixture at 0 °C for 3 h. The reaction solution was concentrated *in vacuo*, and the residue was dissolved in DMF. Chloroacetyl chloride (23 mg, 200 μmol) was added at 0 °C and stirred at 0 °C for 1 h. The reaction solution was concentrated *in vacuo*, and the residue was purified by reverse-phase HPLC using an ODS-3 column and eluted with H_2O/CH_3CN containing 0.1% TFA to yield the title compound (3.8 mg, 2.9 μmol) in 3% yield.

^{1}H NMR (600 MHz; MeOD-d_4): δ 8.18 (br, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.77-7.69 (m, 4H), 7.58 (d, J = 9.0 Hz, 1H), 7.52 (br, 1H), 7.28 (d, J = 9.0 Hz, 2H), 7.07 (dd, J = 9.0, 1.8 Hz, 1H), 6.96 (d, J = 1.8 Hz, 2H), 4.38 (s, 2H), 3.91-3.85 (m, 2H), 3.68 (q, J = 7.2 Hz, 8H), 3.66-3.38 (m, 22H), 3.21 (q, J = 7.2 Hz, 2H), 3.12 (s, 2H), 2.35 (br, 2H), 2.19 (br, 2H), 1.59-1.57 (m, 4H), 1.32-1.28 (m, 15H). **^{13}C NMR (150 MHz, MeOD-d_4)**: δ 176.5, 172.5, 172.3, 167.0, 159.4, 157.6, 157.1, 157.0, 136.6, 133.5, 133.2, 132.8, 131.1, 128.9 (q, $^2J_{C\text{-}F}$ = 31.5 Hz), 128.0 (q, $^3J_{C\text{-}F}$ = 3.0 Hz), 126.1, 126.0 (q, $^3J_{C\text{-}F}$ = 3.0 Hz), 124.9, 123.7 (q, $^1J_{C\text{-}F}$ = 280 Hz), 101.4, 71.6, 71.2, 71.2, 71.2, 70.6, 70.4, 61.0, 53.2, 47.0, 44.2, 40.5, 35.0, 33.3, 25.8, 12.9, 9.25. **HRMS (ESI+):** Calcd for $[M+H]^+$ 1275.5398, found 1275.5382.

Synthesis of PCAF-SPis



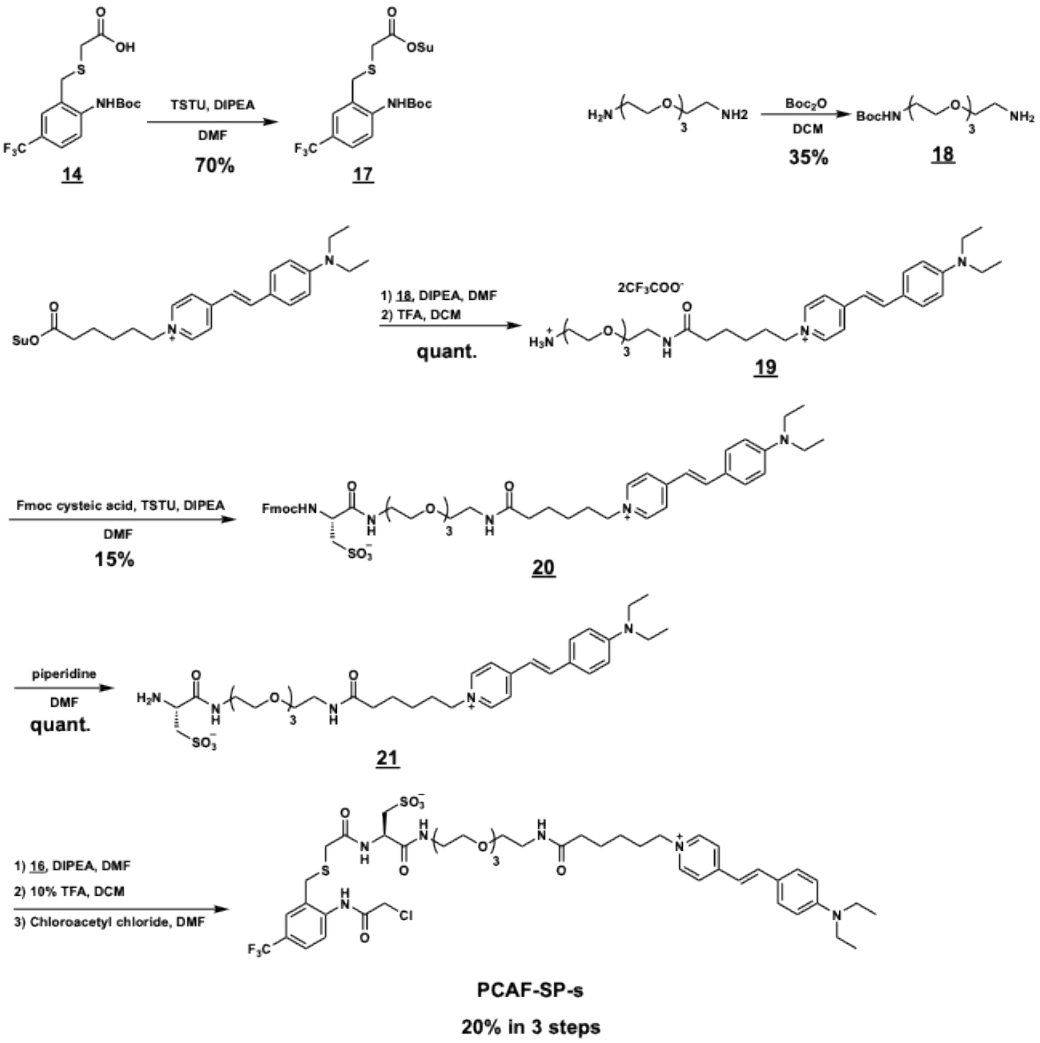
PCAF-SPis

DIPEA (26 mg, 200 μmol) and compound **11** (30 mg, 84 μmol) were dissolved in DMF (5 mL). HBTU (48 mg, 126 μmol) was added to the resulting solution and stirred at 0 °C for 45 min. The crude product **16** was added to the reaction mixture, warmed to room temperature, and stirred overnight. The reaction solution was concentrated *in vacuo*. Then, we added 10% TFA in DCM to the residue and stirred it for 2 h at room temperature. The reaction solution was concentrated *in vacuo*, then the residue was dried for several hours, and dissolved in DMF. Chloroacetyl chloride (23 mg, 200 μmol) was added to the solution that was stirred for 1 h at 0 °C. The reaction solution was concentrated *in vacuo*, then the residue was purified by reverse-phase HPLC using ODS-3 column and eluted with H_2O/CH_3CN containing 0.1% TFA to yield the title compound (3.1 mg, 2.7 μmol) with a 12% overall yield.

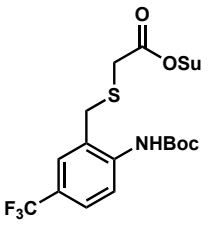
^{1}H NMR (600 MHz, DMSO-d_6): δ 9.95 (br, 1H), 8.73 (d, J= 6.6 Hz, 2H), 8.53 (d, J= 7.2 Hz, 1H), 8.16 (t, J= 5.4 Hz, 1H), 8.04 (d, J= 7.2 Hz, 2H), 7.95 (d, J= 9.0 Hz, 1H), 7.90 (d, J= 16.8 Hz, 1H), 7.84 (t, J= 5.4 Hz, 1H), 7.71 (d, J= 1.8 Hz, 1H), 7.65 (dd, J= 9.0, 1.8 Hz, 1H), 7.56 (d, J= 9.0 Hz, 2H), 7.12 (d, J= 16.8 Hz, 1H), 6.75 (d, J= 9.0 Hz, 2H), 4.76-4.72 (m, 1H), 4.41 (s, 2H), 4.39 (t, J= 7.2 Hz, 2H), 3.88 (dd, J= 34.2, 14.2 Hz, 1H), 3.49-3.15 (m, 22H), 3.11 (s, 2H), 2.07 (t, J= 6.6 Hz, 2H), 1.91-1.84 (m, 2H), 1.55-1.50 (m, 2H), 1.26-1.20 (m, 2H), 1.13 (t, J= 7.2 Hz, 1H),) **^{13}C NMR (150 MHz, MeOD-d_4)**: δ 175.8, 172.2, 171.1, 170.9, 168.2, 156.3, 144.4, 140.6, 132.1, 132.0, 128.8 (q, $^3J_{C\text{-}F}$ = 3.0 Hz), 128.5 (q, $^2J_{C\text{-}F}$ = 30.0 Hz), 126.1, 126.0, 124.8 (q, $^1J_{C\text{-}F}$ = 277 Hz), 101.39, 71.6, 71.2, 70.6, 70.4, 61.0, 53.2, 44.2, 41.8, 40.6, 40.3, 36.5,

35.0, 33.2, 31.8, 27.3, 26.6, 26.1, 12.7. **HRMS (ESI+):** Calcd for $[M]^+$ 1003.4237, found 1003.4215.

1.5 Synthesis of PCAF-SP-s



Compound 17



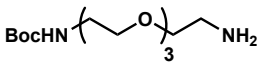
17

TSTU (74.2 mg, 246 μmol), compound **14** (60.3 mg, 164 μmol), and DIPEA (21.2 mg, 164 μmol) were dissolved in DMF (11 mL) and stirred at room temperature for 2 h. The reaction solution was concentrated *in vacuo*, then the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 70/30 as eluent) to yield compound **17** (53.2 mg, 115 μmol) with a 70% overall yield.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (d, *J* = 8.0 Hz, 1H), 7.55-7.54 (m, 2H), 7.31 (br, 1H), 3.99

(s, 2H), 3.40 (s, 2H), 2.89 (s, 4H), 1.53 (s, 9H). **^{13}C NMR (125 MHz, $CDCl_3$):** δ 168.7, 165.5, 152.7, 140.4, 128.0 (q, $^3J_{C\text{-}F}$ = 3.8 Hz), 126.0 (q, $^3J_{C\text{-}F}$ = 3.8 Hz), 125.2 (q, $^2J_{C\text{-}F}$ = 33.8 Hz), 124.3, 124.0 (q, $^1J_{C\text{-}F}$ = 270.0 Hz), 121.7, 81.2, 32.9, 29.6, 28.3, 25.6. **HRMS (ESI+):** Calcd for $[M+Na]^+$ 485.0965, found 485.0985.

Compound <u>18</u>

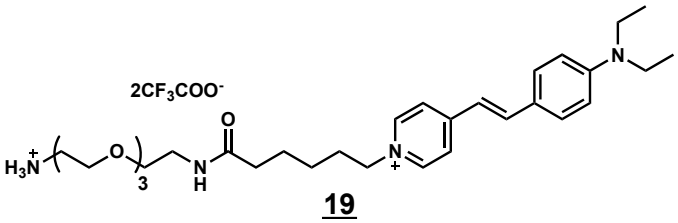


<u>18</u>

1,11-Diamino-3,6,9-trioxaundecane (100 mg, 520 μmol) and Boc_2O (56.2 mg, 260 μmol) were dissolved in DCM (5 mL) on ice and stirred at room temperature overnight. The reaction mixture was washed with water and brine. The solution was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography on silica gel (DCM/MeOH = 99/1 to 70/30 as eluent) to yield compound **<u>18</u>** (27 mg, 92 μmol) with a 35% overall yield.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.31 (br, 1H), 3.67-3.61 (m, 8H), 3.55-3.49 (m, 4H), 3.32 (m, 2H), 2.87 (m, 9H), 1.44 (s, 9H). **^{13}C NMR (125 MHz, $CDCl_3$):** δ 156.1, 79.1, 73.4, 70.6, 70.5, 70.3, 70.2, 41,7. 40.4, 28.4. **HRMS (FAB+):** Calcd for $[M+H]^+$ 293.1998, found 293.2079.

Synthesis of compound **<u>19</u>**



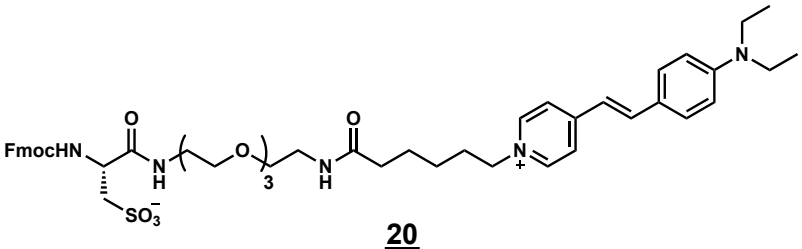
<u>19</u>

Styrylpyridinium-NHS (52 mg, 86 μmol), compound **<u>18</u>** (25.2 mg, 86.1 μmol), and DIPEA (50 μL, 26 μmol) were dissolved in DMF (5.7 mL) and stirred at room temperature for 2.5 h. After removing the solvent, DCM was added to the residue, which was then washed with NH_4Cl aq. and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography using silica gel (DCM to DCM/MeOH = 80/20 as eluent). Then, to the residue (56 mg) we added TFA (590 μL, 7.70 mmol) in DCM (8 mL), and the solution was stirred at room temperature for 6 h and evaporated to yield the title compound (58 mg, 86 μmol, quant.).

^{1}H NMR (500 MHz, MeOD-d_4): δ 8.66 (d, J = 7.0 Hz, 2H), 8.05 (d, J = 7.0 Hz, 2H), 7.88 (d, J = 16 Hz, 1H), 7.74 (d, J = 8.0 Hz, 2H), 7.24 (d, J = 16 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 4.47 (t, J = 7.5 Hz, 2H), 3.71-3.51 (m, 18H), 3.35 (t, J = 5.5 Hz, 2H), 3.12 (t, J = 4.5 Hz, 2H), 2.23

(t, *J* = 7.5 Hz, 2H), 2.03-1.97 (m, 2H) ,1.68 (tt, *J* =7.5 Hz, 2H) ,1.40 (tt, *J* =7.5 Hz, 2H) ,1.19 (t, *J*=7.5 Hz, 6H). **^{13}C NMR (125 MHz, MeOD-d_4):** δ 174.5, 155.1, 150.2, 143.2, 142.8, 130.7, 122.1, 122.0, 115.6, 111.3, 70.1, 70.1, 69.8, 69.7, 69.2, 44.1, 39.2 38.8, 35.1, 30.5, 25.2, 24.7, 11.5. **HRMS (FAB+):** Calcd for [M]+ 541.3748, found 541.3775.

Synthesis of compound **20**

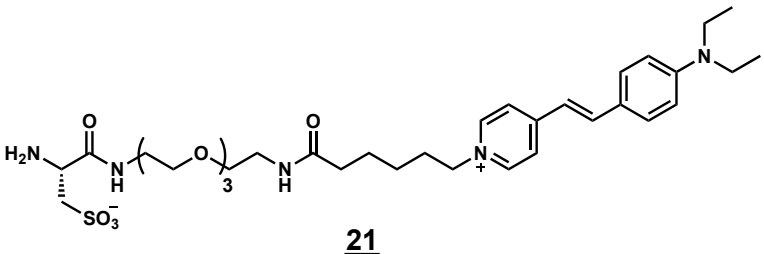


20

Fmoc-cysteic acid (102 mg, 258 μmol), TSTU (96.3 mg, 310 μmol), and DIPEA (135 μL, 775 μmol) were dissolved in DMF (8 mL) at room temperature and stirred at room temperature for 5 h. Compound **19** (140 mg) in DMF (9 mL) was added to the reaction solution and stirred at room temperature overnight. The reaction solution was concentrated *in vacuo*, then the residue was purified by flash column chromatography on silica gel (DCM containing 0.1% AcOH/MeOH containing 0.1% AcOH = 97/3 to 80/20 as eluent) to yield compound **20** (35 mg, 38 μmol) with a 15% overall yield.

^{1}H NMR (500 MHz, MeOD-d_4): δ 8.53 (d, *J* = 7.0 Hz, 2H), 7.90 (d, *J* = 7.0 Hz, 2H), 7.78-7.75 (m, 3H), 7.64 (t, *J* = 8.0 Hz, 2H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.36 (t, *J* = 7.0 Hz, 2H), 7.28 (td, *J* = 7.0 Hz, 1.0 Hz, 2H), 7.49 (d, *J* = 16 Hz, 1H), 6.62 (d, *J* = 8.0 Hz, 2H), 4.53 (m, 1H), 4.38 (t, *J* = 7.5 Hz, 2H), 4.34-4.24 (m, 2H), 4.18 (t, *J* =7.0 Hz, 1H), 3.57-3.16 (m, 22H), 2.22 (t, *J* =7.5 Hz, 2H), 1.95 (tt, *J*=7.5 Hz, 2H), 1.66 (tt, *J* = 7.5 Hz, 2H), 1.35 (tt, *J* =7.5 Hz, 2H), 1.19 (t, *J* =7.0 Hz, 6H). **HRMS (FAB+):** Calcd for $[M]^+$ 914.4368, found 914.4368.

Synthesis of compound **21**

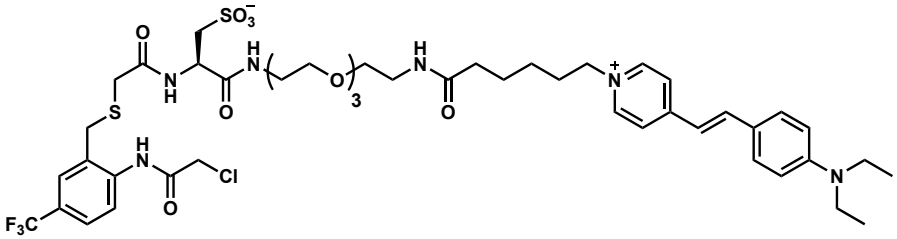


21

Piperidine (320 μL, 3.2 mmol) and compound **20** (37 mg, 40 μmol) were dissolved in DMF (2 mL) and stirred at room temperature for 5 h. The reaction solution was concentrated *in vacuo* and the residue was purified by reverse-phase column chromatography on silica gel

(H_2O/CH_3CN = 80/20 to CH_3CN as eluent) to yield compound **21** (34 mg, quant.).
^{1}H NMR (500 MHz, MeOD-d_4): δ 8.56 (d, J = 7.0 Hz, 2H), 7.95 (d, J = 7.0 Hz, 2H), 7.81 (d, J = 16 Hz, 1H), 7.58 (d, J = 9.0 Hz, 2H), 7.04 (d, J = 16 Hz, 1H), 6.75 (d, J = 9.0 Hz, 2H), 4.42 (t, J = 7.5 Hz, 2H), 3.64-3.33 (m, 20H), 3.19-3.14 (m, 2H), 2.24 (t, J = 7.5 Hz, 2H), 1.98 (tt, J = 7.5 Hz, 2H), 1.67 (tt, J = 7.5 Hz, 2H), 1.35 (m, 2H), 1.20 (t, J =7.0 Hz, 6H).**^{13}C NMR (125 MHz, MeOD-d_4):** δ 174.5, 155.0, 150.2, 143.1, 142.9, 130.7, 122.2, 122.0, 115.7, 111.3, 70.1, 69.9, 69.8, 691, 68.7, 59.5, 50.7, 50.5, 44.1, 39.4, 38.9, 35.1, 30.4, 25.1, 24.7, 11.5. **HRMS (FAB+):** Calcd for $[M]^+$ 692.3688, found 692.3696.

Synthesis of **PCAF-SP-s**

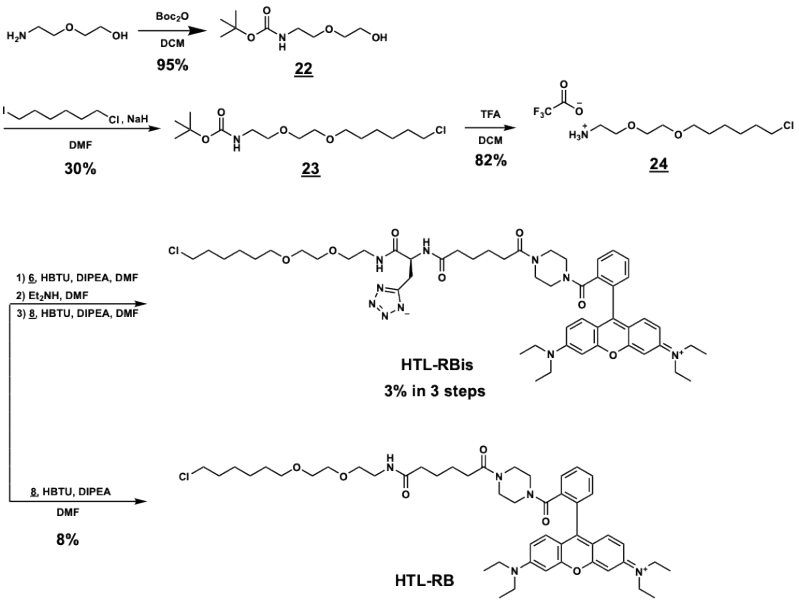


PCAF-SP-s

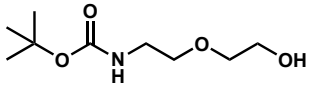
DIPEA (19 µL, 110 µmol), compound **21** (37 mg, 54 µmol), and compound **17** (26 mg, 54 µmol) were dissolved in DMF (3 mL) and stirred at room temperature for 9 h. The reaction solution was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (DCM to DCM/MeOH = 20/80 as eluent) to yield the crude product (35.4 mg). TFA (3 mL) was added dropwise to the solution of the crude product in DCM (5 mL) at 0 °C. The solution was stirred for 3.5 h at room temperature and concentrated *in vacuo*. The resulting residue was dissolved in DCM (4 mL). Chloroacetyl chloride (6.5 µL, 41 µmol) and TEA (14 µL, 100 µmol) in DCM (65 µL) were added to the solution that was stirred for 3.5 h at room temperature. The solution was concentrated *in vacuo*, then the residue was purified by reverse-phase HPLC using an ODS-3 column and eluted with H_2O/CH_3CN containing 0.1% TFA to yield **PCAF-SP-S** (11 mg, 11 µmol) with a 20% overall yield.
^{1}H NMR (500 MHz, MeOD-d_4) : δ 8.65 (d, J = 6.5 Hz, 2H), 8.02 (d, J = 6.5 Hz, 2H), 7.94 (d, J = 8.5 Hz, 1H), 7.85 (d, J = 16.0 Hz, 1H), 7.71-7.68 (m, 3H), 7.54 (dd, J = 8.5, 2.0 Hz, 1H), 7.48 (d, J = 16.0 Hz, 1H), 7.01 (br, 2H), 4.77 (dd, J = 4.0 Hz, 1H), 4.46 (t, J = 7.0 Hz, 2H), 4.43 (s, 2H), 3.98 (s, 2H), 3.63-3.20 (m, 22H) , 2.24 (t, J = 7.0 Hz, 2H), 1.99 (tt, J = 7.5 Hz, 2H), 1.68 (tt, J =7.5 Hz, 2H), 1.41-1.35 (m, 2H), 1.19 (t, J = 7.5 Hz, 6H). **^{13}C NMR (125 MHz, MeOD-d_4):** δ 175.9, 172.5, 171.9, 168.3, 155.7, 144.8, 142.9, 140.6, 132.0, 131.8, 128.6 (q, $^3J_{C\text{-}F}$ = 3.8 Hz), 128.3 (q, $^2J_{C\text{-}F}$ = 32.5 Hz), 126.0, 125.9 (q, $^3J_{C\text{-}F}$ = 3.8 Hz), 125.4 (q, $^1J_{C\text{-}F}$ = 270 Hz), 124.5, 120.8, 117.0, 71.6, 71.5, 71.3, 71.2, 70.5, 70.4, 61.2, 52.9, 52.7, 44.4, 40.7, 40.3, 36.4, 35.7, 33.2, 31.7,

26.4, 26.0, 12.1. **HRMS (FAB+):** Calcd for $[M]^+$ 1015.3682, found 1015.3692.

1.6 Synthesis of **HTL-RBis** and **HTL-SPis**



Synthesis of compound 22

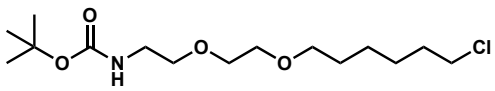


22

A solution of Boc_2O (6.35 g, 30.0 mmol) in DCM (35 mL) was slowly added to a solution of 2-(2-aminoethoxy)-ethanol (3.18 g, 30.0 mmol) in DCM (20 mL) at 0 °C. The reaction solution was stirred at 0 °C for 30 min and warmed to room temperature overnight. The reaction mixture was washed with water and the aqueous layer was extracted with DCM (3× 50 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to obtain the title compound as a colorless oil (5.86 g, 28.6 mmol) in 95% yield. The product was sufficiently pure and used without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ 5.08 (br, 1H, H), 3.76-3.73 (m, 2H), 3.58 (t, *J*= 4.2 Hz, 2H), 3.56 (t, *J*= 4.2 Hz, 2H), 3.34-3.33 (m, 2H), 2.59 (s, 1H), 1.45 (s, 9H).**^{13}C NMR (150 MHz, $CDCl_3$)**: δ 156.2, 79.5, 72.3, 70.4, 61.8, 40.4, 28.5. **HRMS (ESI+):** Calcd for $[M+Na]^+$ 228.1206, found 228.1209

Synthesis of compound 23



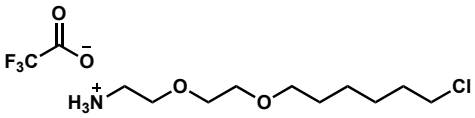
23

A suspension of NaH (60% in oil, 267 mg, 6.5 mmol) in dry hexane was washed with dry hexane

three times and then the excess hexane was removed. A solution of compound **22** (1.03 g, 5.0 mmol) in dry THF was slowly added to the suspension at 0 °C and stirred for 30 min under N_2. The reaction mixture was warmed to room temperature and stirred overnight. We added 10 mL of aqueous 10% NH_4Cl and EtOAc, and the aqueous layer was extracted three times with 100 mL EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using silica gel column chromatography (hexane/ EtOAc = 55/45 to 30/70 as eluent) to yield compound **23** as a colorless oil (491 mg, 1.52 mmol) with a 30% overall yield.

^{1}H NMR (600 MHz, $CDCl_3$): δ 5.02 (br, 1H), 3.62-3.53 (m, 8H), 3.47 (t, *J*= 6.0 Hz, 2H), 3.33-3.32 (m, 2H), 1.81-1.76 (m, 2H), 1.64-1.59 (m, 2H), 1.49-1.46 (m, 2H), 1.40-1.35 (m, 2H), 1.44 (s, 9H).**^{13}C NMR (150 MHz, $CDCl_3$)**: δ 156.1, 79.2, 71.3, 70.3, 70.1, 45.1, 40.4, 32.6, 29.5, 28.5, 26.8, 25.5. **HRMS (ESI+):** Calcd for $[M+Na]^+$ 346.1756, found 346.1749.

Synthesis of compound 24

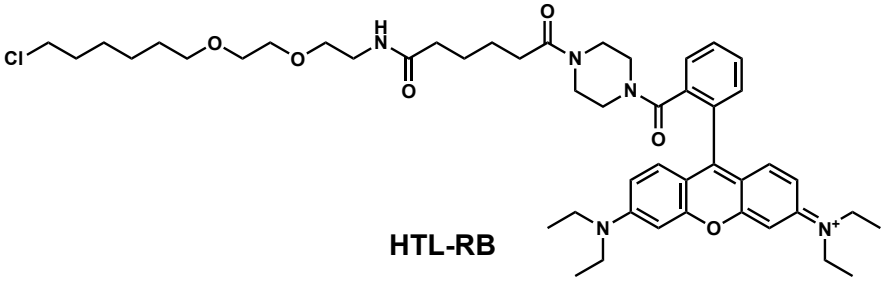


24

TFA (1 mL) was added to a solution of compound **23** in DCM (4 mL) and stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo* and the resultant solution was purified by silica gel column chromatography (DCM/MeOH = 99/1 to 20/80 as eluent) to yield compound **24** (239 mg, 0.709 mmol) with an 82% overall yield.

^{1}H NMR (600 MHz, $CDCl_3$): δ 5.73 (br, 3H), 3.68-3.57 (m, 6H), 3.53 (t, *J*= 7.2 Hz, 2H), 3.45 (t, *J*= 7.2 Hz, 2H), 3.15 (m, 2H), 1.78-1.74 (m, 2H), 1.60-1.55 (m, 2H), 1.46-1.41 (m, 2H), 1.36-1.30 (m, 2H).**^{13}C NMR (150 MHz, $CDCl_3$)**: δ 162.5 (q, *J*= 149.4 Hz ^{13}C-F coupling), 116.4 (q, *J*= 1160 Hz ^{13}C-F coupling), 71.3, 70.1, 69.7, 66.5, 53.8, 45.2, 39.8, 32.5, 29.1, 26.6, 25.2. **HRMS (ESI+):** Calcd for $[M+H]^+$ 224.1412, found 224.1417.

Synthesis of HTL-RB



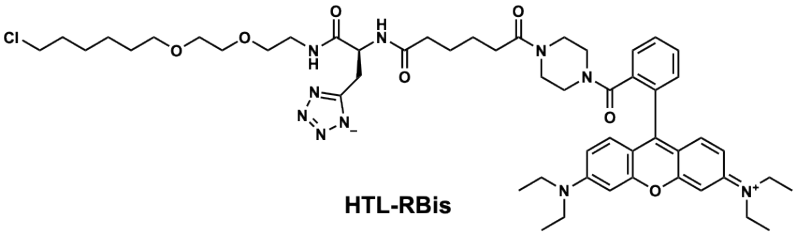
HTL-RB

DIPEA (129 mg, 1.00 mmol) and compound **8** (32 mg, 0.050 mmol) were dissolved in DMF (8

mL). HBTU (38 mg, 0.10 mmol) was added to the resulting solution, which was stirred at 0 °C for 45 min. We added compound **24** (86 mg, 0.26 mmol) in DMF (2 mL) to the reaction mixture. The solution was warmed to room temperature and stirred overnight. After removing the solvent, the residue was purified by reverse-phase HPLC using an ODS-3 column and eluted with H_2O/CH_3CN containing 0.1% TFA to yield the title compound (3.5 mg, 4.2 μmol) with an 8% overall yield.

^{1}H NMR (600 MHz, DMSO-d_6): δ 7.82-7.53 (m, 4H), 7.15 (d, J = 9.0 Hz, 2H), 7.11 (dd, J = 9.0 Hz, J = 1.8 Hz, 2H), 6.95 (d, J = 1.8 Hz, 2H), 3.65 (q, J = 9.6 Hz, 8H), 3.63-3.16 (m, 21H), 2.25 (t, J = 9.6 Hz, 2H), 2.07-2.05 (m, 2H), 1.73-1.67 (m, 2H), 1.51-1.26 (m, 10H), 1.20 (t, J = 9.6 Hz, 12H).**^{13}C NMR (150 MHz, DMSO-d_6)**: δ 172.6, 171.2, 157.6, 156.1, 155.6, 135.8, 132.3, 131.3, 131.0, 130.3, 128.0, 114.8, 113.6, 96.4, 70.7, 70.1, 70.0, 69.7, 45.9, 45.9, 39.0, 32.5, 29.6, 26.6, 25.5, 13.0. **HRMS (FAB+):** Calcd for $[M]^+$ 844.4774, found 844.4776.

Synthesis of HTL-RBis



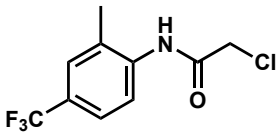
HTL-RBis

DIPEA (129 mg, 1.00 mmol) and compound **6** (126 mg, 0.330 mmol) were dissolved in DMF (8 mL). HBTU (150 mg, 0.396 mmol) was added to the resulting solution at 0 °C and stirred at 0 °C for 45 min. We added compound **24** (134 mg, 0.396 mmol) in DMF (2 mL) to the reaction mixture, which was warmed to room temperature and stirred overnight. The solution was concentrated *in vacuo*. Then we added 10% Diethyl amine in DMF (3 mL) to the residue and stirred the mixture at room temperature for 4 h. After removing the solvent, we added DIPEA (129 mg, 1.00 mmol) and HBTU (75 mg, 0.170 mmol) in DMF (2 mL) to the residue, and stirred the mixture at 0 °C for 45 min. We added compound **24** (86 mg, 0.26 μmol) in DMF (2 mL) to the reaction mixture at 0 °C, warmed the solution to room temperature, and stirred it overnight. The solution was concentrated *in vacuo*, and the residue was purified by reverse-phase HPLC using an ODS-3 column and eluted with H_2O/CH_3CN containing 0.1% TFA to yield the title compound (4.3 mg, 4.4 μmol) with a 3% overall yield.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.09 (br, 1H), 7.97 (br, 1H), 7.77-7.71 (m, 3H), 7.53 (br, 1H), 7.15 (d, J = 9.6 Hz, 2H), 7.11 (dd, J = 9.6 Hz, J = 1.8 Hz, 2H), 6.95 (d, J = 1.8 Hz, 2H), 4.68-4.67 (m, 2H), 3.65 (q, J = 9.6 Hz, 8H), 3.63-3.16 (m, 24H), 2.22 (t, J = 9.6 Hz, 2H), 2.08-2.07 (m, 2H), 1.71-1.66 (m, 2H), 1.51-1.26 (m, 10H), 1.20 (t, J = 9.6 Hz, 12H).**^{13}C NMR (150 MHz, DMSO-d_6)**: δ 172.6, 171.2, 170.4, 157.6, 156.1, 155.6, 135.8, 132.3, 131.3, 131.0, 130.3, 128.0, 114.8,

113.6, 96.4, 70.7, 70.1, 69.9, 69.3, 51.5, 45.9, 45.9, 39.0, 32.5, 29.6, 26.6, 25.5, 13.0. **HRMS (ESI+):** Calcd for $[M]^+$ 983.5268, found 983.5289.

PCAF-Me



PCAF-Me

We prepared **PCAF-Me** as structurally similar model compounds of PCAF probes (**PCAF-RB**, **PCAF-RBis**, **PCAF-SP**, **PCAF-SPis**, **PCAF-SP-s**) for the determination of the C-F coupling constants in ^{13}C NMR of the PCAF probes.

^{1}H NMR (600 MHz, MeOD-d_4): δ 7.70 (d, J = 8.4 Hz, 1H), 7.55 (s, 1H), 7.50 (d, J = 8.4 Hz, 1H), 4.28 (s, 2H), 2.35 (s, 3H). **^{13}C NMR (150 MHz, MeOD-d_4)**: δ 168.0, 140.1, 134.4, 129.1 (q, $^2J_{C\text{-}F}$ = 31.5 Hz), 128.5 (q, ^3J = 3.0 Hz), 126.3, 126.0 (q, ^1J = 270 Hz), 124.4 (q, ^3J = 3.0 Hz), 43.7, 17.9.

2. Chemical Biology Procedures

Plasmid construction

pcDNA3.1(+)-MBP-PYP^{WT}

Plasmid construction was performed as previously described.[S2]

pcDNA3.1(+)-PYP^{WT}-EGFR

Plasmid construction was performed as previously described.[S3]

pcDNA3.1(+)-HA-PYP^{WT}-NLS

Plasmid construction was performed as previously described.[S4]

pcDNA3.1(+)-HA-PYP^{NQN}-NLS

Plasmid construction was performed as previously described.[S5]

pcDNA3.1(+)-HA-Halo-NLS

Plasmid construction was performed as previously described.[S5]

Preparation of recombinant proteins (PYP-tag)

Escherichia coli cells [BL21 (DE3) (Novagen)] transformed with plasmids encoding the PYP-tag (PYP^{WT}, PYP^{NQN}) were cultivated in Luria-Bertani medium containing 100 μg/mL of ampicillin at 37 °C until the OD_{600} value of the culture medium reached 0.6–0.8. At this point, the temperature was lowered to 20 °C with the addition of IPTG (Isopropyl β-D-1-thiogalactopyranoside). Then, overnight protein expression was induced overnight to afford a final protein concentration of 100 μM. The cells were harvested by centrifugation at 5,000 rpm for 15 min, resuspended in binding buffer (50 mM sodium phosphate, 300 mM NaCl, 1 mM DTT, pH 8.0), and lysed by sonication. The cell lysate supernatant was obtained by centrifugation at 15,000 rpm for 20 min and passed through a Ni column (Roche). The resin was washed with a wash buffer (50 mM sodium phosphate, 300 mM NaCl, 5 mM imidazole, and 1 mM DTT; pH 8.0) and eluted with a second buffer (50 mM sodium phosphate, 300 mM NaCl, 250 mM imidazole, and 1 mM DTT; pH 8.0) in accordance with the manufacturer's protocol. The eluted fraction was further purified through size exclusion chromatography (SuperdexTM 75 10/300 GL, GE Healthcare) using a running buffer (20 mM HEPES and 150 mM NaCl; pH 7.4) and the purity and molecular weight of proteins were assessed by SDS-PAGE. The purified protein was dissolved in the assay buffer (20 mM HEPES and 150 mM NaCl; pH 7.4) and then flash-frozen

with liquid nitrogen for storage at –80 °C

Protein labeling reactions *in vitro* for SDS-PAGE analysis

PYP-tag (1.0 μM) and the probes (2.0 μM) were incubated in the assay buffer (20 mM HEPES, pH 7.4, containing 150 mM NaCl) at 37 °C for 30 min. The reaction was then quenched with *N*-ethylmaleimide (f.c. 25 mM) at room temperature for 5 min. The reaction solutions were heated at 103 °C for 5 min and subsequently analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Fluorescence images were obtained using Gel mie-ru mini470 (Wako Pure Chemical Industries). The gel was stained with Coomassie Brilliant Blue. The excitation wavelength used for fluorescence imaging was 470 nm.

Absorption spectroscopy

Absorption spectra were measured after the probe (5.0 μM) was incubated with or without PYP-tag (10 μM) in the assay buffer (20 mM HEPES, pH 7.4, containing 150 mM NaCl) at 37 °C for 30 min.

Fluorescence spectroscopy

Fluorescence spectra were measured after the probe (5.0 μM) was incubated with or without PYP-tag (10 μM) in the assay buffer at 37 °C for 30 min. The fluorescence spectra of the mixtures were recorded at an excitation wavelength of 484 nm (**PCAF-SP**, **PCAF-SPis**, and **PCAF-SP-s**) or 535 nm (**PCAF-RB** and **PCAF-RBis**), with a slit width of 5.0 nm for both excitation and emission. Relative fluorescence quantum yields were determined using fluorescein in 0.1 M NaOH aq. (Ex: 470 nm, Φ_{fl} = 91%)[S6] and Rhodamine B in water (Ex: 535 nm, Φ_{fl} = 36%)[S7] as a reference.

Kinetic analyses of protein labeling reactions

The fluorescence half-life $t_{1/2}$ and second-order rate constant k_2 of the labeling reaction of each probe were obtained from time-course experiments. Each probe (1.0 μM) was reacted with PYP-tag (2.0 μM) in the assay buffer (20 mM HEPES, pH 7.4, containing 150 mM NaCl) at 37 °C and the fluorescence intensity was monitored with the slit width of 5.0 nm. The fluorescence half-lives and second-order rate constants were calculated using the following equations:

$$F_t = ((\exp(k_2\, t_{1/2}([A]_0 - [B]_0)) - 1) / (\exp(k_2\, t_{1/2}([A]_0 - [B]_0)) - [B]_0 / [A]_0))\,(F_{max} - F_0) + F_0 \quad \text{Equation 1}$$

$[A]_0$ and $[B]_0$ represent the initial concentrations of [PYP-tag] and [Probe], respectively. F_t and F_0 represent the observed fluorescence intensity at time t and at the initial point, respectively. F_{max}

was determined using the Equation 1.

Cell cultures

HEK293T cells were cultured in DMEM containing 10% fetal bovine serum and PS as antibiotics (100 U/mL penicillin and 0.1 mg/mL streptomycin). Transfected cells were maintained in a 5% CO_2 atmosphere at 37 °C throughout the experiments.

Fluorescence imaging of proteins in living cells using PCAF-RB or PCAF-RBis

HEK293T cells were transfected with pcDNA3.1(+)-PYP^{NQN}-NLS using Lipofectamine 3000 (Invitrogen), according to the manufacturer's protocol. After incubating the transfected cells in 5% CO_2 at 37 °C for 24 h, the cells were washed with HBSS buffer three times. Then, the cells were incubated with 5.0 μM probe in DMEM for 30 min. The cells were incubated for an additional 10 min after replacing the medium with DMEM. Cell images were acquired with a cell washing step (three times with HBSS buffer) using a confocal laser scanning microscope. All images were captured at an excitation frequency of 561 nm with an emission range of 566–685 nm using a 63× lens (Zeiss LSM 880).

Localization analysis of PCAF-RB in living cells using MitoTracker Green FM

HEK293T cells were washed thrice with HBSS buffer. The cells were incubated with 100 nM **PCAF-RB** and 100 nM MitoTracker Green FM in DMEM for 60 min. Cell images were acquired using a washing step (three times with HBSS buffer). All images were captured at excitation frequencies of 470/40 nm and 560/40 nm with emission ranges of 525/50 and 560/40 nm, respectively, using a 60× lens (Keyence BZ-X710).

Fluorescence imaging of proteins in living cells using PCAF-SP and PCAF-SPis

HEK293T cells were transfected with pcDNA3.1(+)-PYP^{WT}-NLS using Lipofectamine 3000 (Invitrogen), according to the manufacturer's protocol. After incubation of the transfected cells in 5% CO_2 at 37 °C for 24 h, the cells were washed with HBSS buffer three times. Then, the cells were incubated with 1.25 μM probe in DMEM for 60 min. Cell images were acquired without the cell washing step using a confocal laser scanning microscope. All images were captured at an excitation frequency of 488 nm and an emission range of 550–650 nm using a 63× lens (Zeiss LSM 880).

Fluorescence imaging of proteins in living cells using PCAF-SP-s and PCAFred

HEK293T cells were transfected with pcDNA3.1(+)-PYP^{WT}-NLS and PYP^{WT}-EGFR or empty

vector using Lipofectamine 3000 (Invitrogen), according to the manufacturer's protocol. After incubation of the transfected cells in 5% CO_2 at 37 °C for 24 h, the cells were washed with HBSS buffer three times. Then, the cells were incubated with 1.0 μM **PCAF-SP-s** and 1.0 μM **PCAFred** in DMEM for 30 min. Cell images were acquired without the cell washing step using a confocal laser scanning microscope. All images were obtained with excitation at 488 nm and 633 nm by detecting 500-600 (green) and 660–710 (magenta) nm emissions, respectively, using a 63× lens (Zeiss LSM 880).

Fluorescence imaging of proteins in living cells using PCAF-SP-s

HEK293T cells were transfected with pcDNA3.1(+)-MBP-PYP^{WT}, PYP^{WT}-NLS, PYP^{WT}-EGFR, or empty vector using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol. After incubating the transfected cells in 5% CO_2 at 37 °C for 24 h, the cells were washed with HBSS buffer three times. Then, the cells were incubated with 1.0 μM **PCAF-SP-s** in DMEM for 30 min. Cell images were acquired without the cell washing step using a confocal laser scanning microscope. All images were captured at an excitation frequency of 473 nm with an emission range of 490–590 nm using a 60× lens (Olympus, Fv10i).

Comparative labeling efficiency of PYP-tag with PCAF-SP and PCAF-SPis

HEK293T cells were transfected with pcDNA3.1(+)-PYP^{WT}-NLS using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol. After incubation of the cells at 37 °C for 24 h, the cells were washed with HBSS three times. The cells were incubated with 10 μM **PCAF-SP** and **PCAF-SPis,** respectively, in DMEM for 4 h. After the incubation, the cells were centrifuged at 500 g for 5 min at 4 °C and removed the supernatant. The resultant cells were lysed in the lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% (v/v) Triton X-100) on ice for 15 min. The cell lysate was centrifuged at 15000 g for 15 min at 4 °C. The HA-PYP fusion protein was isolated from the supernatant using the HA-tagged Protein Purification Kit (Medical & Biological Laboratories Co., Ltd.) according to the manufacturer's protocol. The sample was heated at 95 °C for 5 min and subsequently analyzed by SDS–PAGE. Fluorescence images were obtained using Gel mie-ru mini470 (Wako Pure Chemical Industries). The gel was stained with Coomassie Brilliant Blue. The excitation wavelength used for fluorescence imaging was 470 nm.

Live-cell imaging of GLUT4 with insulin stimulation using PCAF-SP-s

Stable HeLa cell lines expressing PYP-$GLUT4^{WT}$ were cultured in DMEM supplemented with 10% FBS. The medium was replaced with Krebs-Ringer bicarbonate (KRB) buffer, and the cells were incubated for 3 h. Following incubation with KRB buffer, insulin (100 nM) was added to

the cells and incubated for 20 min. After insulin stimulation, **PCAF-SP-s** (1.5 μM) was added to the cells and incubated for 10 minutes. After washing the cells with KRB buffer three times, the medium was replaced with FluoroBrite DMEM and all images were captured at an excitation frequency of 488 nm with an emission range of 500–630 nm using a 63× lens (Zeiss LSM 880).

Live-cell imaging of intracellular GLUT4 using PCAF-RBis

Stable HeLa cell lines expressing PYP-GLUT4^{WT} were cultured in DMEM supplemented with 10% FBS. The medium was replaced with Krebs-Ringer bicarbonate (KRB) buffer, then the cells were incubated for 3 h. Following the incubation of KRB buffer, **PCAF-RBis** (1.25 μM) was added to the cells and incubated for 20 min. After washing the cells three times with KRB buffer, the medium was replaced with FluoroBrite DMEM, and all images were captured at an excitation frequency of 561 nm with an emission range of 635–705 (red), using a 63× lens (Zeiss LSM 880).

Immunostaining of intracellular PYP-GLUT4^{WT} using anti-FLAG and labeling with PCAF-RBis

Stable HeLa cell lines expressing PYP-GLUT4^{WT} were incubated in KRB buffer for three hours, and then incubated with **PCAF-RBis** (1.25 μM) for 20 min. After washing the cells three times with PBS, and then incubated with formaldehyde solution on ice for 15 min. The cells were washed three times with PBS, incubated with 0.1% Triton for 5 minutes at room temperature, and then washed with PBS. The cells were treated with 5% BSA for 30 minutes, and then incubated with anti-FLAG antibody as primary antibody in PBS with 3% BSA for an hour at room temperature. After washing with PBS for 5 min (3x), the cells were incubated with secondary antibody (anti-mouse conjugated Alexa647) in PBS with 3% BSA for an hour at room temperature. All images were captured using excitation frequencies of 561 nm and 633 nm with emission ranges of 570-620 nm (orange) and 640-700 nm (magenta), respectively, using 63x lens (Zeiss LSM 880).

Immunostaining of PYP-GLUT4^{WT} using anti-FLAG and labeling with PCAF-SP-s

Stable HeLa cell lines expressing PYP-GLUT4^{WT} were cultured in DMEM supplemented with 10% FBS. The medium was replaced with Krebs-Ringer bicarbonate (KRB) buffer, and the cells were incubated for 3 h. Following incubation with KRB buffer, insulin (100 nM) was added to the cells and incubated for 20 min. After insulin stimulation, **PCAF-SP-s** (2.5 μM) was added to the cells and incubated for 10 minutes. After washing the cells three times with PBS, and then incubated with formaldehyde solution on ice for 15 min. The cells were washed three times with PBS, incubated with 0.1% Triton for 5 minutes at room temperature, and then washed with PBS.

The cells were treated with 5% BSA for 30 minutes, and then incubated with anti-FLAG antibody as primary antibody in PBS with 3% BSA for an hour at room temperature. After washing with PBS for 5 min (3x), the cells were incubated with secondary antibody (anti-mouse conjugated Alexa647) in PBS with 3% BSA for an hour at room temperature. All images were captured using excitation frequencies of 561 nm and 633 nm with emission ranges of 500-620 nm (green) and 640-700 nm (magenta), respectively, using 63x lens (Zeiss LSM 880).

Multicolor imaging for visualization of multiple localization of GLUT4 labeled with PCAF-SP-s and PCAF-RBis

Stable HeLa cell lines expressing PYP-GLUT4^{WT} were cultured in DMEM supplemented with 10% FBS. The medium was replaced with Krebs-Ringer bicarbonate (KRB) buffer, and the cells were incubated for 3 h. Following incubation with KRB buffer, insulin (100 nM) was added to the cells and incubated for 20 min. After insulin stimulation, **PCAF-SP-s** (1.5 μM) was added to the cells and incubated for 10 min. The medium was replaced with KRB buffer without insulin and the cells were incubated for 20 min. **PCAF-RBis** (1.25 μM) was added to the cells and incubated for 20 min. After washing the cells with KRB buffer three times, the medium was replaced with FluoroBrite DMEM, and all images were captured using excitation frequencies of 488 nm and 561 nm with emission ranges of 500–630 (green) and 635–705 (red) nm, respectively, using a 63× lens (Zeiss LSM 880).

Fluorescence imaging of proteins in living cells using HTL-RB or HTL-RBis

HEK293T cells were transfected with pcDNA3.1(+)-PYP^{NQN}-NLS using Lipofectamine 3000 (Invitrogen), according to the manufacturer's protocol. After incubation of the transfected cells in 5% CO_2 at 37 °C for 24 h, the cells were washed with HBSS buffer three times. Then, the cells were incubated with 2.5 μM probe in DMEM for 60 min. After replacing the medium with DMEM, the cells were incubated for an additional 30 min and then rinsed 3 × 5 min in DMEM before image acquisition. All images were captured at an excitation frequency of 561 nm with an emission range of 566–685 nm using a 63× lens (Zeiss LSM 880).

Supplementary Text S1

To evaluate labeling efficiency, we modified a method that we previously reported.[S1] In this method, HA-tag fused PYP-NLS was labeled with probe (in this work; **PCAF-SP** or **PCAF-SPis**), and purified using anti-HA tag conjugated beads. Then, the samples were subjected to SDS-PAGE. Since the band mobility of labeled proteins shifts compared to that of unlabeled proteins, labeling efficiency can be estimated by the degree of band shift. In the absence of the probes, we observed intense and weak CBB bands, one detected in the lower position and the other in the upper, respectively (Fig. S4a lane 1). One probable reason for this phenomenon is the presence of posttranslational processing such as *N*-terminal deletion, since labeling reactions also gave two fluorescent bands, which indicate that both are derived from PYP proteins. The CBB band was shifted from the lower position to the upper upon labeling reactions and was overlapped with the unlabeled band in the upper position (Fig. S4a lane 3 and 5). We thus decided to determine the intensity ratio (the upper band to the lower) of both the CBB bands, whose increment is the estimated efficiency of the labeling reactions (Fig. S4b). The ratio increased by approximately 10% and 20% upon the addition of **PCAF-SPis** and **PCAF-SP**, respectively, compared to unlabeled protein (**SPis**: 0.44→0.53, **SP**: 0.44→0.62). These results indicate that the labeling efficiency of **PCAF-SP** is higher than that of **PCAF-SPis**, likely due to the higher cell membrane permeability of **PCAF-SP** compared to **PCAF-SPis**.

Supplementary Text S2

According to a previous GLUT4 imaging study,[S1] HeLa cells stably expressing PYP^{WT}-GLUT4, which was fused to FLAG-tag, can be observed with immunostaining using anti-FLAG antibody. We thus analyzed the localization of GLUT4 not only by fluorescence labeling with the probes but also by immunostaining with the antibodies (primary antibody: anti-FLAG mouse antibody, secondary antibody: Alexa Fluor 647 conjugated anti-mouse antibody). As shown in the 1st row in Fig. S8, the fluorescence derived from GLUT4 labeled with **PCAF-SP-s** in the cell membrane overlaps with that obtained using the antibodies. As a control experiment, we conducted single labeling or staining experiments (**PCAF-SP-s** or antibody) (the 2nd and the 3rd row in Fig. S7) to confirm clean separation of each emission channel. The fluorescence of **PCAF-SP-s** was not observed in the image of HeLa cells that do not express PYP-tag (the 4th row in Fig. S7), indicating that **PCAF-SP-s** can be applied for specific labeling and imaging of membrane-localized GLUT4.

Meanwhile, to examine whether **PCAF-RBis** successfully labeled intracellular GLUT4 imaging without off-target effects, we conducted the localization analysis of intracellular GLUT4 without insulin stimulation by dual color imaging using **PCAF-RBis** labeling and immunostaining (Fig. S8). As shown in the 1st row in Fig. S8, the localization of GLUT4 labeled with **PCAF-RBis** overlaps with that observed using the antibodies. We also confirmed that each fluorescence channel was fully separated through the control experiment (the 2nd and the 3rd row in Fig. S8), showing that no bleed-through was observed in each channel.

In HeLa cells expressing PYP-GLUT4, the fluorescence signals from **PCAF-SP-s** were observed not only at the cell membrane but also in the cytoplasm of a few cells. This result suggested that GLUT4 internalization occurred between the insulin wash-out process and fixation, following the labeling of membrane-localized GLUT4 under insulin stimulation with **PCAF-SP-s** that can selectively label cell surface proteins. In contrast, fluorescence signals by immunostaining were not clearly observed in the cytoplasm (Fig. S7), while intracellular GLUT4 was stained by immunostaining in the cells without insulin stimulation (Fig. S8). These results may indicate that insulin stimulation could restrain the recognition of the FLAG-tag by the antibody, possibly because the GLUT4 internalization may cause the interaction of some biomolecules with the intracellular domain of GLUT4 near the epitope sequence. To fully understand these results, further studies are required but beyond the scope of this research.

3. Supplementary Figures

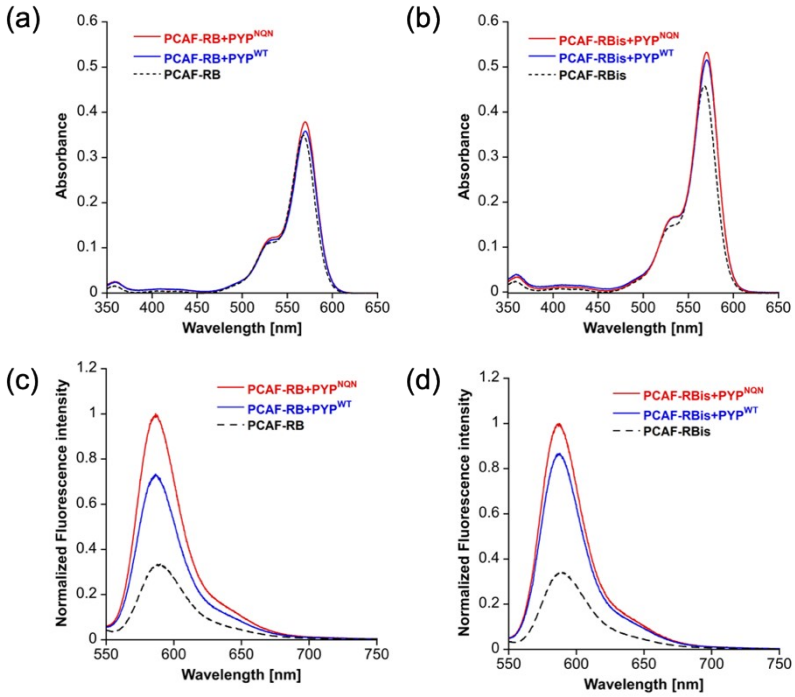


Fig. S1 (a and b) Absorption spectra of 5.0 μM (a) **PCAF-RB** and (b) **PCAF-RBis** in the absence and presence of 10.0 μM PYP^{WT} or PYP^{NQN} in a solution of 20 mM HEPES, 150 mM NaCl, and 0.1% DMSO buffered to pH 7.4 at 37 °C. (c and d) Fluorescence spectra of 0.5 μM (c) **PCAF-RB** or (d) **PCAF-RBis** in the absence and presence of 1.0 μM PYP^{WT} or PYP^{NQN} in a solution of 20 mM HEPES, 150 mM NaCl, and 0.1% DMSO buffered to pH 7.4 at 37 °C. The spectra were obtained with an excitation of 535 nm.

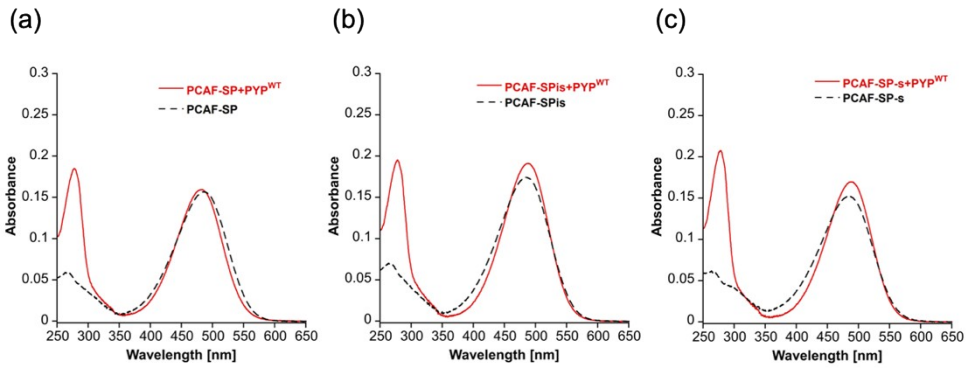


Fig. S2 (a-c) Absorption spectra of 5.0 μM (a) **PCAF-SP**, (b) **PCAF-SPis**, and (c) **PCAF-SP-s** in the absence and presence of 10.0 μM PYP^{WT} in an aqueous solution of 20 mM HEPES, 150 mM NaCl, and 0.1% DMSO buffered to pH 7.4 at 37 °C.

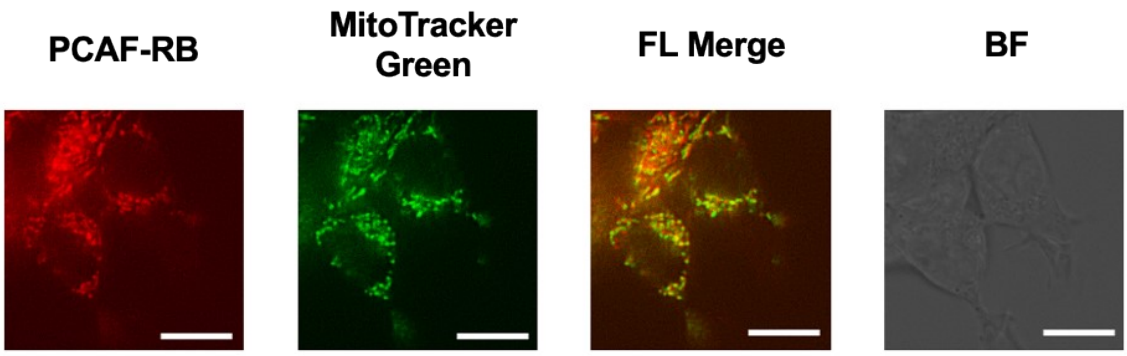


Fig. S3 Localization analysis of **PCAF-RB** (100 nM) in HEK293T cells with MitoTracker Green (100 nM). The images were measured at 560/40 (green) and 630/75 (red) nm emission with the excitation at 470/40 and 525/50 nm, respectively. FL and BF denote the fluorescence image and bright field image, respectively. FL Merge indicates the FL Red image merged with the FL green image. Scale bars, 10

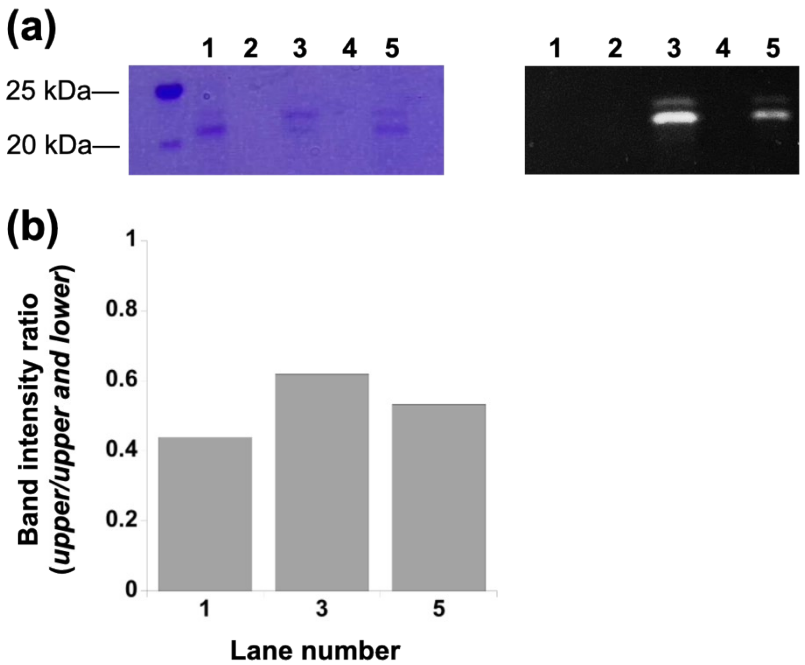


Fig. S4 SDS-PAGE analysis of the probe labeling efficiency in live cells. (a) The CBB and fluorescence images were shown in the left and the right, respectively. The latter was obtained with the excitation at 470 nm. Lane 1: sample of cells expressing HA-PYP-NLS incubated without the probes, Lane 2: sample of non-transfected cells incubated with 10 μM **PCAF-SP**, Lane 3: sample of cells expressing HA-PYP-NLS incubated with 10 μM **PCAF-SP**, Lane 4: sample of non-transfected cells incubated with 10 μM **PCAF-SPis**, Lane 5: sample of cells expressing HA-PYP-NLS incubated with 10 μM **PCAF-SPis** (b) CBB band intensity ratio (upper band/upper and lower bands) in lanes 1, 3, and 5

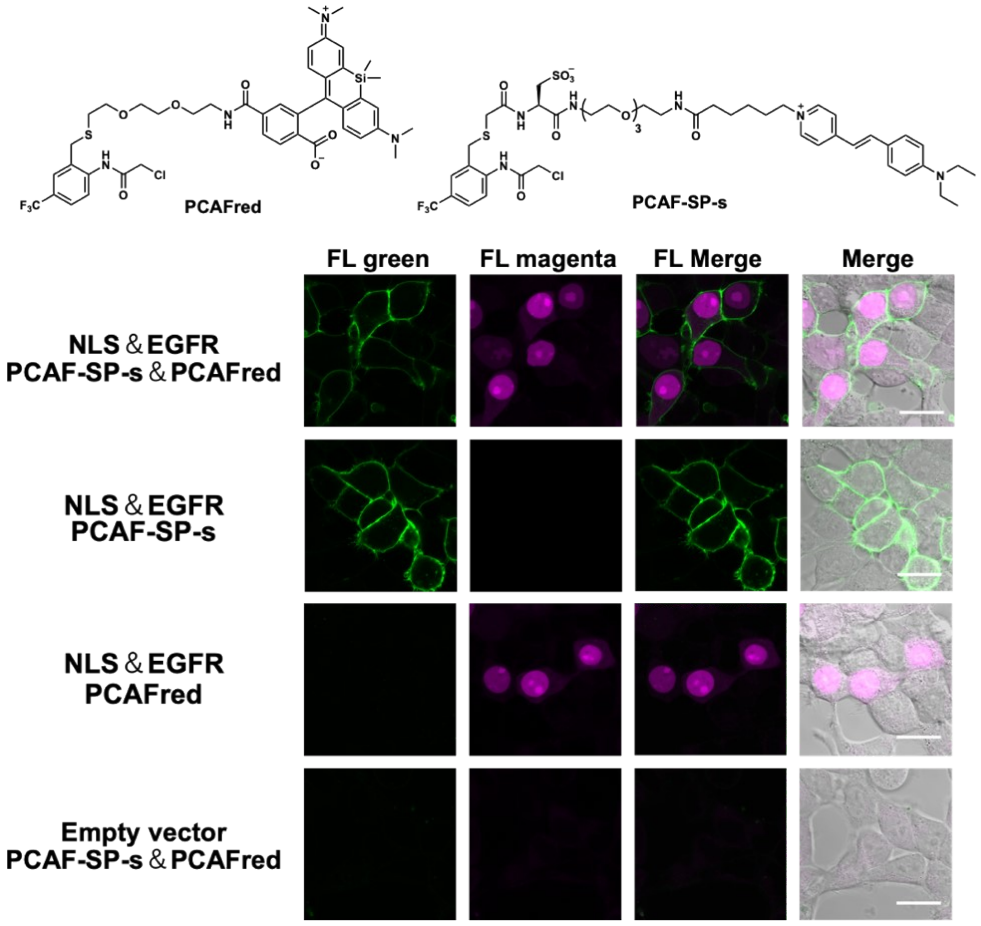


Fig. S5 Fluorescence images of HEK293T cells co-expressing HA-PYP^{WT}-NLS and HA-PYP^{WT}-EGFR or mock cells (transfected with empty vector) labeled with **PCAF-SP-s** (1.0 μM) and **PCAFred**[S1] (1.0 μM). The images were obtained with the excitation at 488 nm and 633 nm by detecting 500–600 (green) and 660–710 (magenta) nm emissions, respectively. Scale bars, 10 μm. FL denotes fluorescence image. FL Merge and Merge denote the magenta fluorescence images merged with that of the green and FL Merge overlayed with phase contrast images, respectively. Experiments were performed twice, giving similar results.

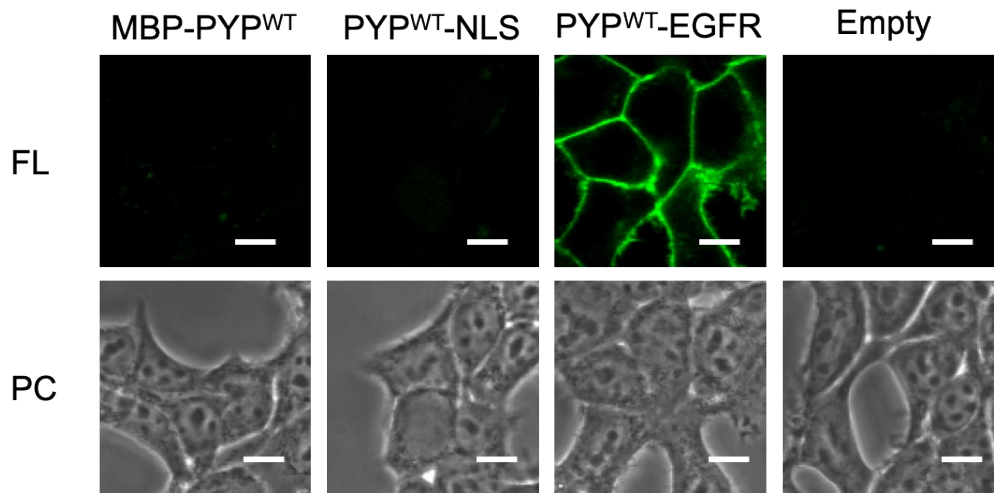


Fig. S6 Fluorescence images and phase contrast images of HEK293T cells expressing HA-PYP^{WT}-NLS, HA-PYP^{WT}-NLS, HA-PYP^{WT}-EGFR, or mock cells (transfected with an empty vector) stained with **PCAF-SP-s** (1.0 μM). The images were measured at a 490–590 nm emission with the excitation at 473 nm. Scale bars, 10 μm. FL denotes the fluorescence image.

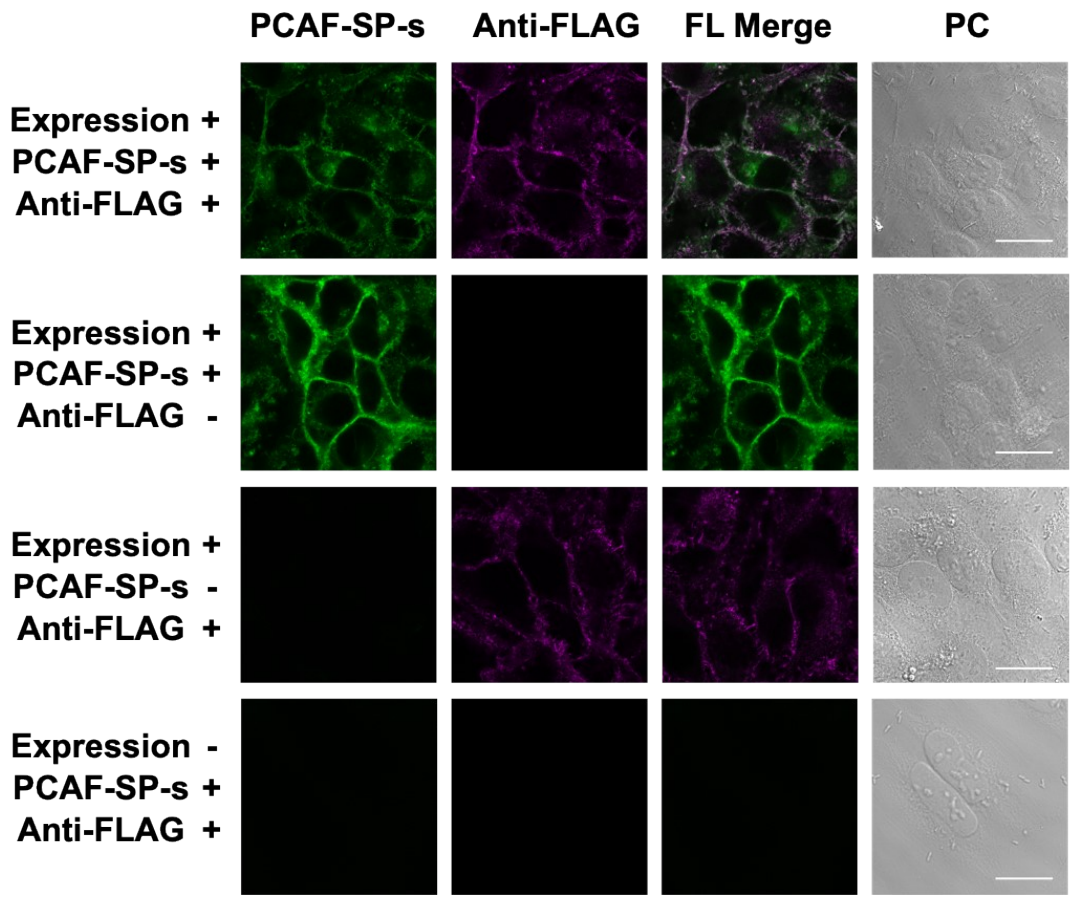


Fig. S7 Multicolor imaging of HeLa cells stably expressing PYP^{WT}-GLUT4 or HeLa cells stained with **PCAF-SP-s** (2.5 μM) and Alexa Fluor 647 conjugated antibody for visualization of GLUT4. All images were obtained with the excitation at 488 and 633 nm and detection at 500–620 (green) and 640–700 (magenta) nm emission, respectively. Scale bars, 20μm. FL and PC denote fluorescence image and phase contrast images, respectively. FL Merge denotes FL green image merged with FL magenta image. Experiments were performed twice, giving similar results.

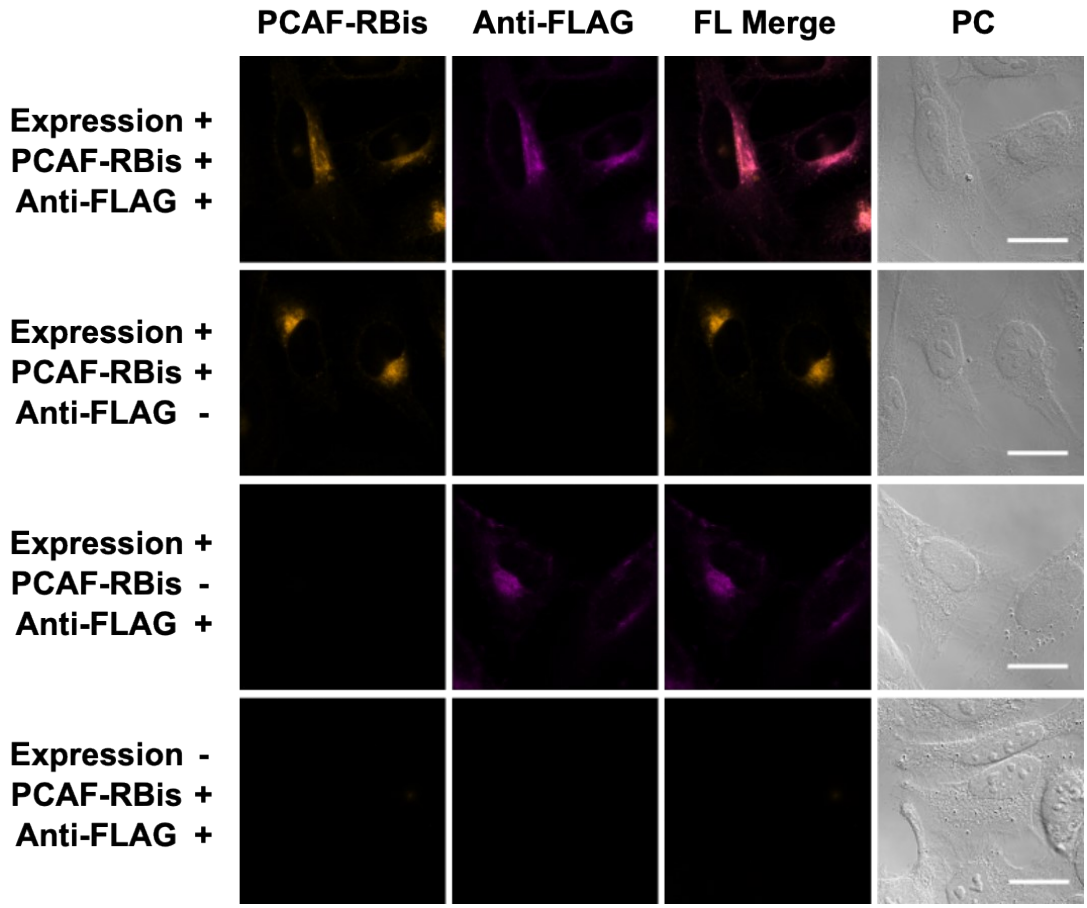


Fig. S8 Multicolor imaging of HeLa cells stably expressing PYP^{WT}-GLUT4 or HeLa cells stained with **PCAF-RBis** (1.25 μM) and Alexa Fluor 647 conjugated antibody for visualization of GLUT4. All images were obtained with the excitation at 561 and 633 nm and detection at 570–620 (orange) and 640–700 (magenta) nm emission, respectively. Scale bars, 20 μm. FL and PC denote fluorescence image and phase contrast image, respectively. FL Merge denotes FL orange image merged with FL magenta image. Experiments were performed twice, giving similar results.

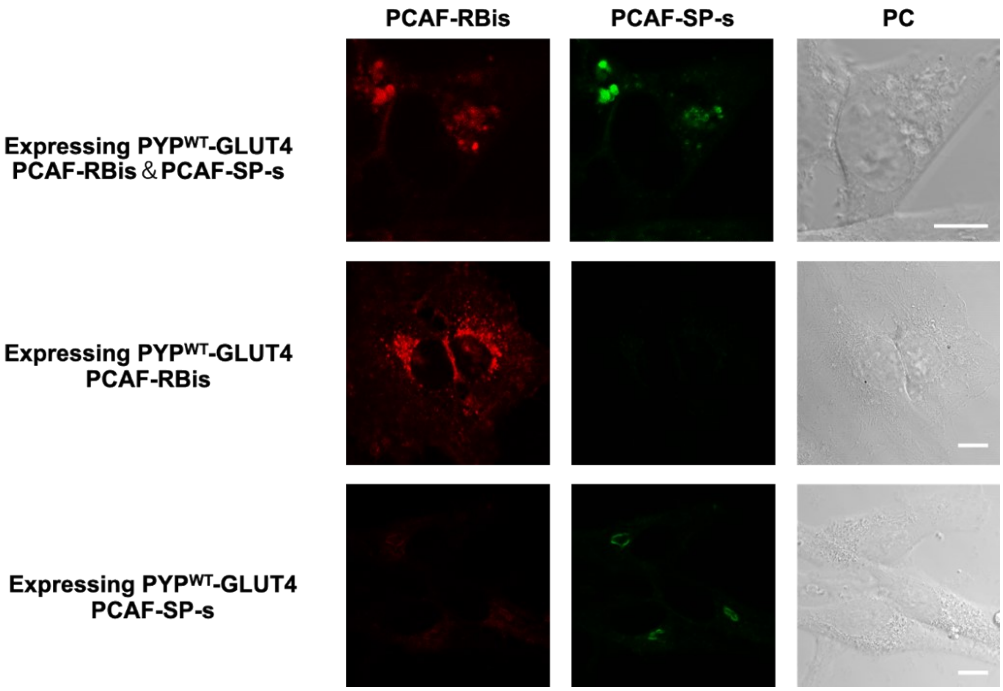


Fig. S9 Multicolor imaging of HeLa cells stably expressing PYP^{WT}-GLUT4 stained with **PCAF-RBis** (1.25 μM) and **PCAF-SP-s** (1.5 μM) for visualization of GLUT4. All images were obtained with the excitation at 488 and 561 nm and detection at 500–630 (green) and 635–705 (red) nm emission, respectively. Scale bars, 10 μm. FL and PC denote fluorescence image and phase contrast image, respectively. FL Merge denotes FL orange image merged with FL magenta image. Experiments were performed twice, giving similar results.

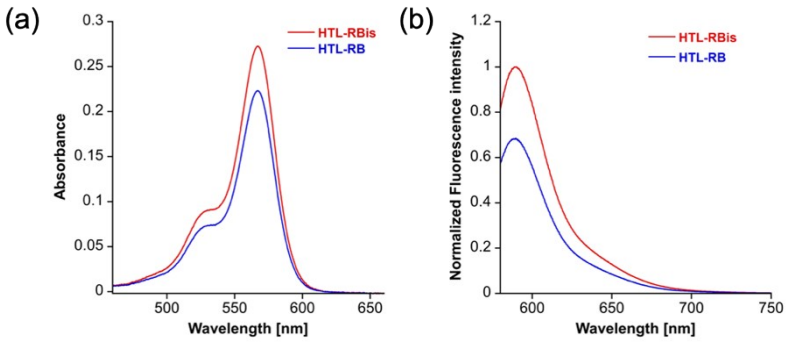


Fig. S10 (a) Absorption spectra of 5.0 μM **HTL-RBis** and **HTL-RBis**. (b) Fluorescence spectra of 5.0 μM **HTL-RBis** and **HTL-RBis**. These spectra were measured in aqueous solution (20 mM HEPES, 150 mM NaCl, and 0.1% DMSO) buffered to pH 7.4 at 37 °C. The spectra were obtained with the excitation at 567 nm.

4. Supplementary Table

Table S1. Photophysical properties and labeling kinetics of PYP-tag labeling probes

Probe	λ_{abs} [nm]	ε[a] [M^{-1} cm^{-1}]	λ_{em} [nm]	Φ_{fl}	k_2[b] [M^{-1} s^{-1}]
PCAF-RBis	568	92000	591	0.29	-
PCAF-RBis+PYP^{WT}	571	100000[c]	587	0.61	3.4 (±0.5) x 10^3
PCAF-RBis+PYP^{NQN}	570	110000[c]	587	0.70	7.0 (±0.7) x 10^3
PCAF-RB	568	70000	590	0.28	-
PCAF-RB+PYP^{WT}	570	72000[c]	587	0.54	6.3 (±0.7) x 10^3
PCAF-RB+PYP^{NQN}	570	76000[c]	586	0.69	5.7 (±0.9) x 10^3
PCAF-SPis	484	35000	610	0.01	-
PCAF-SPis+PYP^{WT}	487	38000[c]	578	0.28	9.1 (±0.9) x 10^3
PCAF-SPis+PYP^{NQN}	487	30000[c]	579	0.29	4.1 (±0.4) x 10^3
PCAF-SP-s	484	31000	609	0.01	-
PCAF-SP-s+PYP^{WT}	487	34000[c]	579	0.29	4.4 (±0.2) x 10^3
PCAF-SP-s+PYP^{NQN}	486	34000[c]	578	0.30	3.8 (±0.1) x 10^3
PCAF-SP	484	31000	608	0.01	-
PCAF-SP+PYP^{WT}	486	31000[c]	578	0.27	5.9 (±0.4) x 10^3
PCAF-SP+PYP^{NQN}	481	30000[c]	576	0.25	21 (±1.0) x 10^3

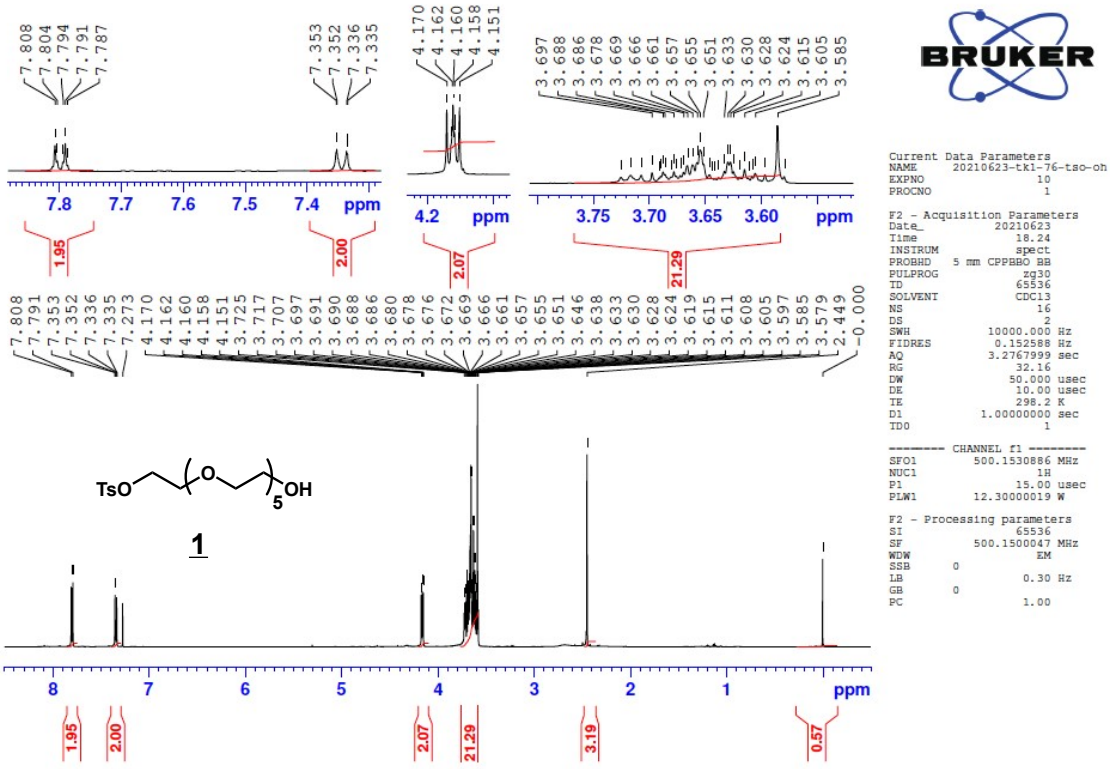
All the experiments were conducted in the solution of 20 mM HEPES, 150 mM, and 0.1% containing DMSO buffered to pH 7.4 at 37 ℃.
[a] ε is extinction coefficient at λ_{abs}.
[b] All data were obtained in triplicate experiments.
[c] Data obtained after labeling reactions of PYP-tag proteins with probes were complete.

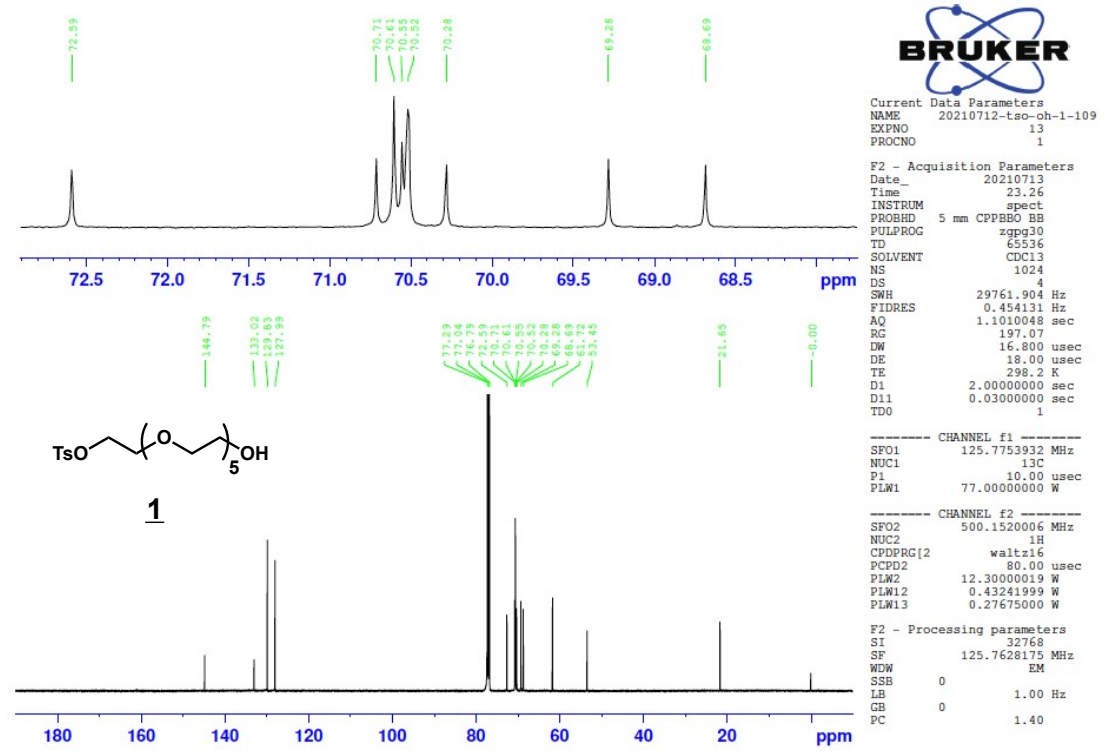
Table S2. Photophysical properties of Halo tag labeling probes

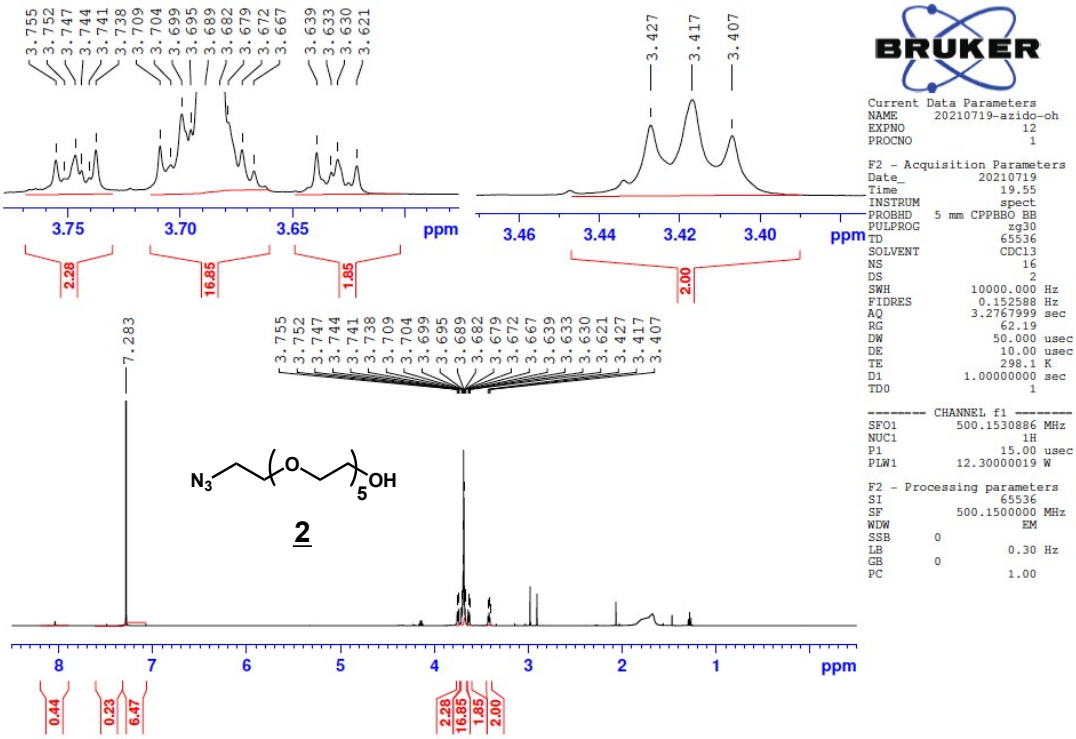
Probe	λ_{abs} [nm]	ε[a] [M^{-1} cm^{-1}]	λ_{em} [nm]	Φ_{fl}
HTL-RBis	567	50000	590	0.24
HTL-RB	567	45000	590	0.20

[a] ε is extinction coefficient at λ_{abs}.

5. NMR Spectra





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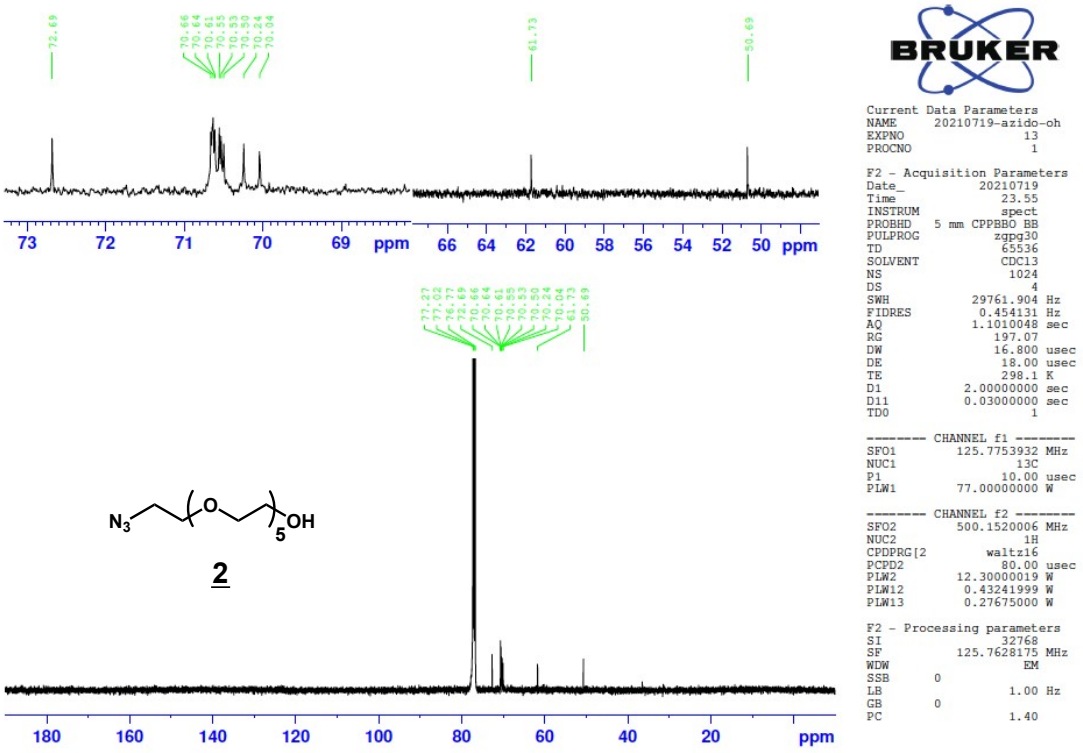


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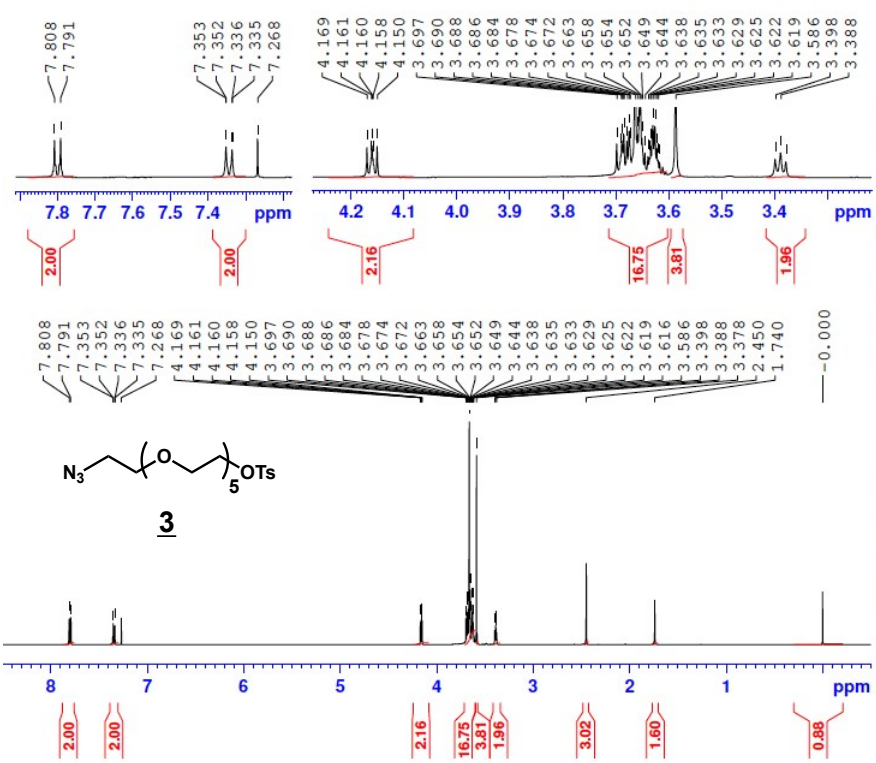

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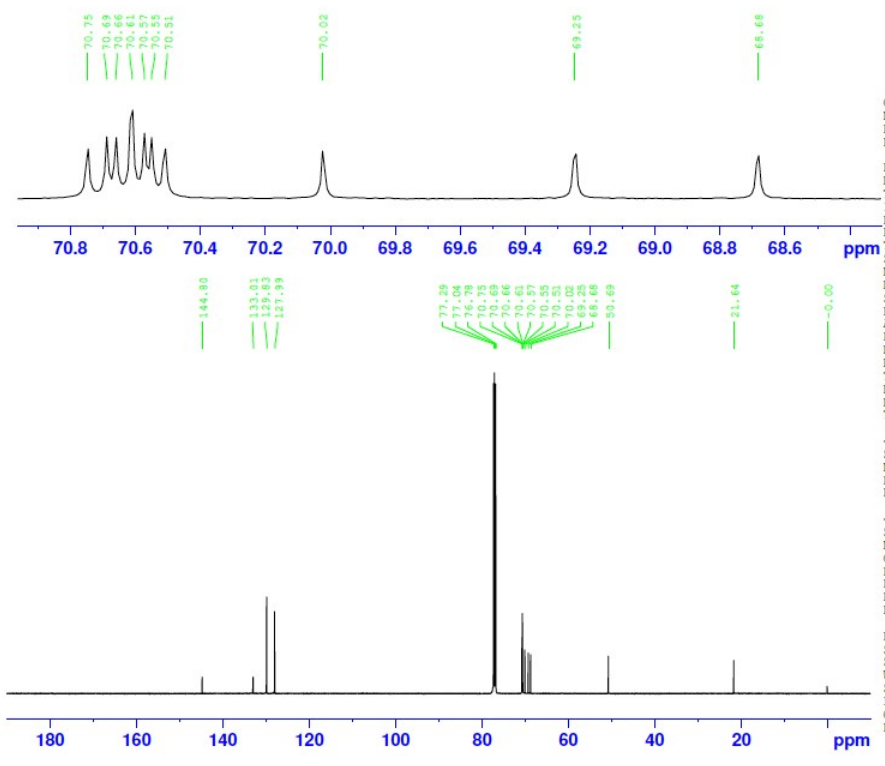

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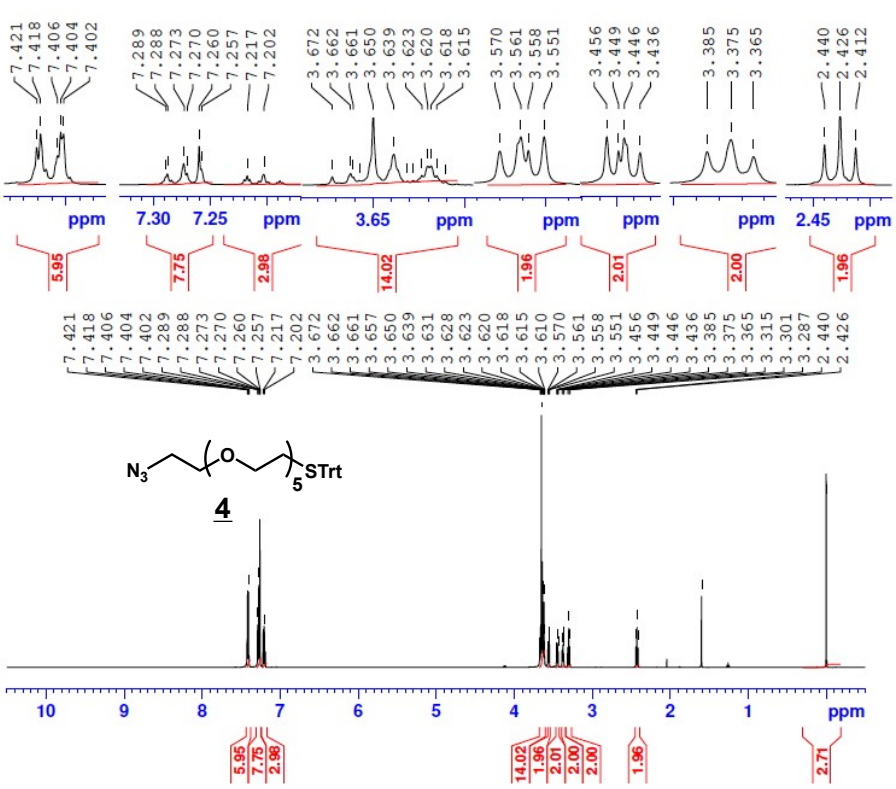
PCPD2             80.00 usec
PLW2         12.30000019 W
PLW12         0.43241999 W
PLW13         0.27675000 W

F2 - Processing parameters
SI                32768
SF           125.7628181 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



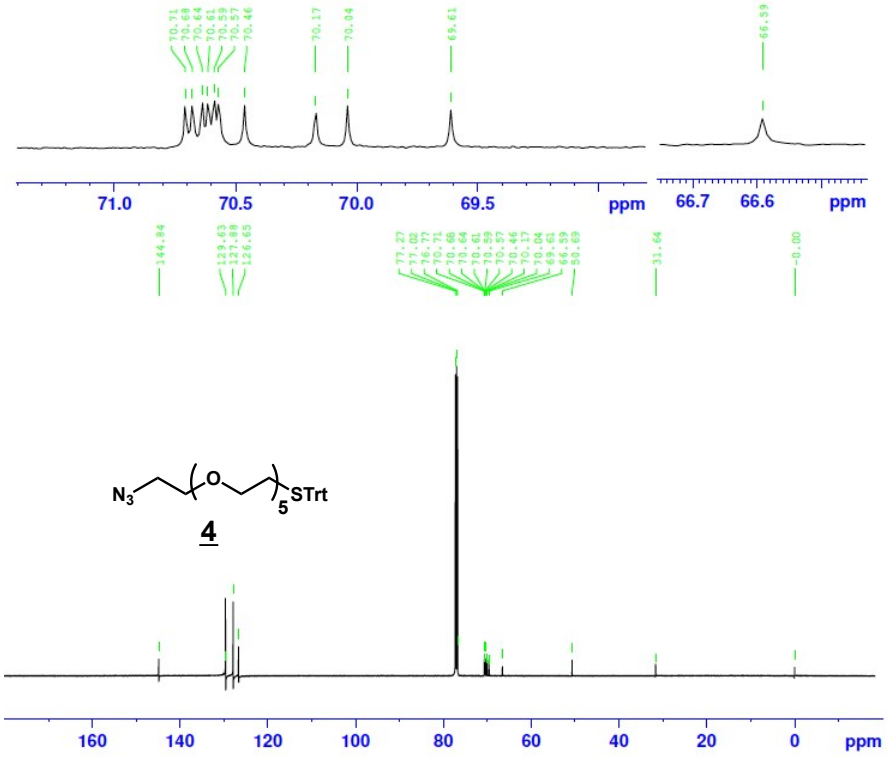
BRUKER

```
Current Data Parameters
NAME      20210916-2-40-fr4-5
EXPNO                      10
PROCNO                      1

F2 - Acquisition Parameters
Date_               20210916
Time                   11.35
INSTRUM                spect
PROBHD   5 mm CPPBBO BB
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH               10000.000 Hz
FIDRES                0.152588 Hz
AQ                 3.2767999 sec
RG                     62.19
DW                    50.000 usec
DE                    10.00 usec
TE                    298.2 K
D1                 1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1            500.1530886 MHz
NUC1                       1H
P1                      15.00 usec
PLW1           12.30000019 W

F2 - Processing parameters
SI                    65536
SF              500.1500115 MHz
WDW                      EM
SSB                   0
LB                     0.30 Hz
GB                    0
PC                     1.00
```



BRUKER

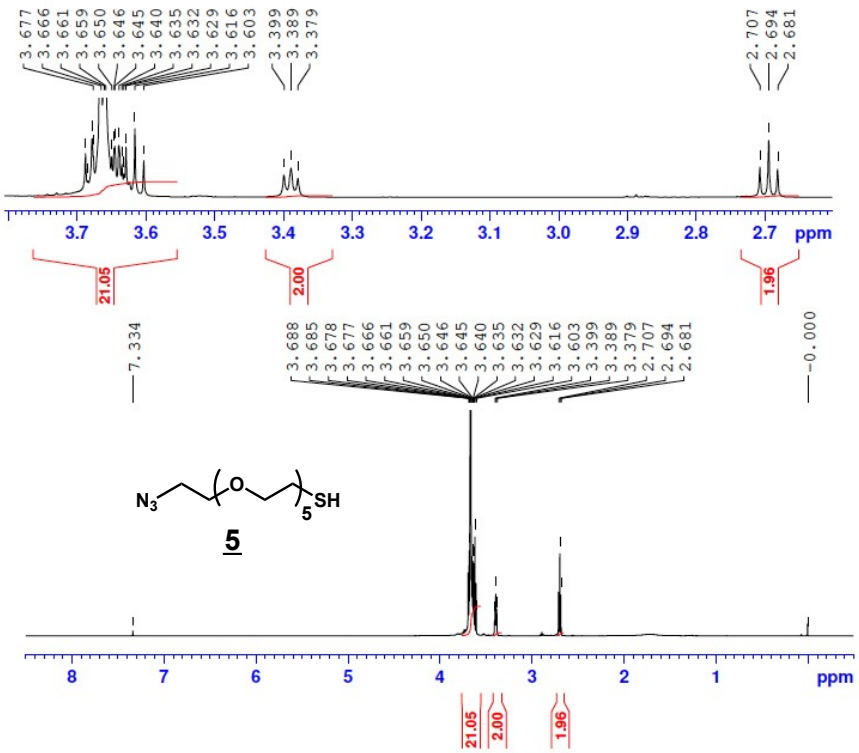
```
Current Data Parameters
NAME      20210916-2-40-fr4-5
EXPNO                      11
PROCNO                      1

F2 - Acquisition Parameters
Date_               20210916
Time                   12.47
INSTRUM                spect
PROBHD   5 mm CPPBBO BB
PULPROG             zgpg30
TD                    65536
SOLVENT               CDCl3
NS                     1024
DS                        4
SWH              29761.904 Hz
FIDRES                0.454131 Hz
AQ                 1.1010048 sec
RG                     197.07
DW                    16.800 usec
DE                    18.00 usec
TE                    298.1 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1           125.7753932 MHz
NUC1                      13C
P1                     10.00 usec
PLW1           77.00000000 W

======== CHANNEL f2 ========
SFO2            500.1520006 MHz
NUC2                       1H
CPDPRG[2                waltz16
PCPD2                  80.00 usec
PLW2           12.30000019 W
PLW12           0.43241999 W
PLW13           0.27675000 W

F2 - Processing parameters
SI                    32768
SF             125.7628181 MHz
WDW                      EM
SSB                   0
LB                     1.00 Hz
GB                    0
PC                     1.40
```

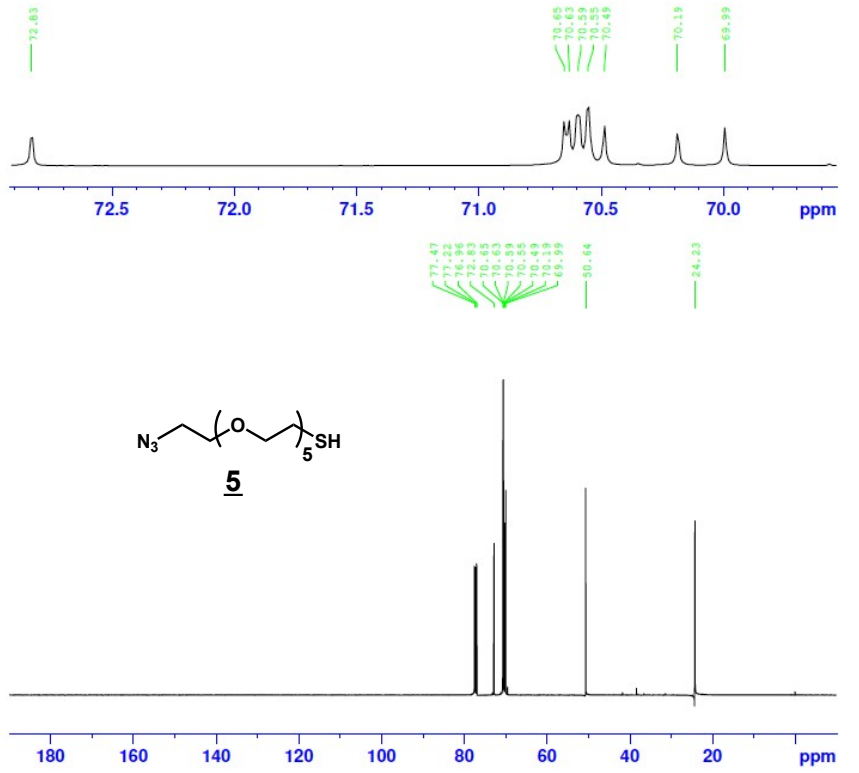


```
Current Data Parameters
NAME      20210716-n3-sh
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210716
Time              17.17
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zg30
TD               65536
SOLVENT           CDC13
NS                   16
DS                    2
SWH           10000.000 Hz
FIDRES        0.152588 Hz
AQ            3.2767999 sec
RG                11.04
DW              50.000 usec
DE              10.00 usec
TE                298.1 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        500.1530886 MHz
NUC1                   1H
P1                 15.00 usec
PLW1        12.30000019 W

F2 - Processing parameters
SI                 65536
SF          500.1499738 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



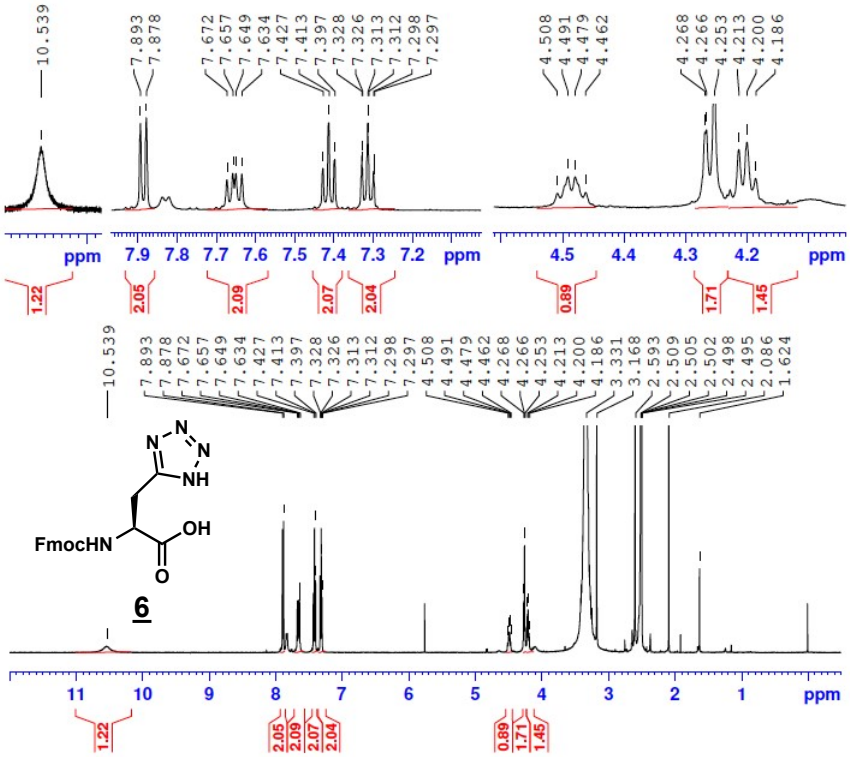
```
Current Data Parameters
NAME      20210716-n3-sh-13C
EXPNO                   10
PROCNO                   1

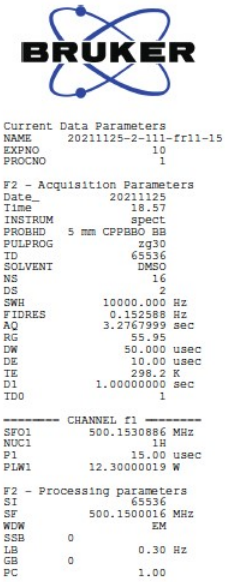
F2 - Acquisition Parameters
Date_             20210716
Time                20.05
INSTRUM             spect
PROBHD     5 mm CPPBBO BB
PULPROG           zgpg30
TD                 65536
SOLVENT            CDC13
NS                  1024
DS                     4
SWH            29761.904 Hz
FIDRES          0.454131 Hz
AQ              1.1010048 sec
RG                 197.07
DW                16.800 usec
DE                18.00 usec
TE                298.1 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

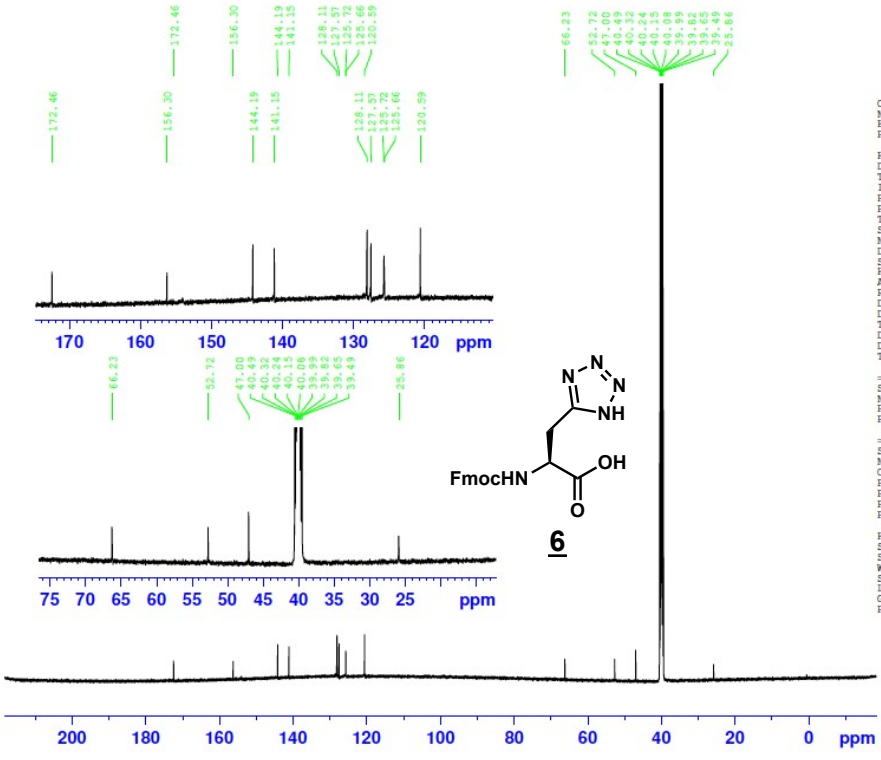
======== CHANNEL f1 ========
SFO1          125.7753932 MHz
NUC1                    13C
P1                  10.00 usec
PLW1          77.00000000 W

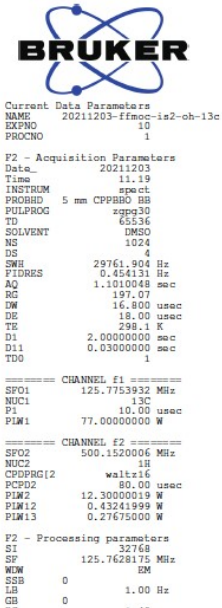
======== CHANNEL f2 ========
SFO2          500.1520006 MHz
NUC2                    1H
CPDPRG[2          waltz16
PCPD2               80.00 usec
PLW2          12.30000019 W
PLW12          0.43241999 W
PLW13          0.27675000 W

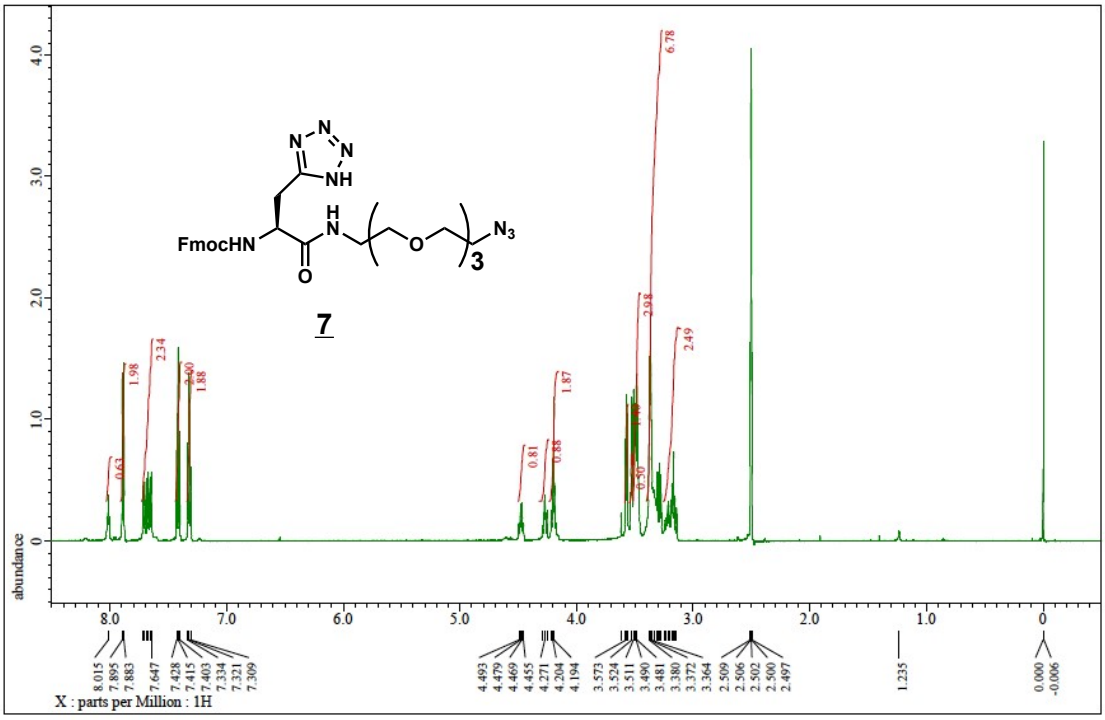
F2 - Processing parameters
SI                  32768
SF          125.7628153 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

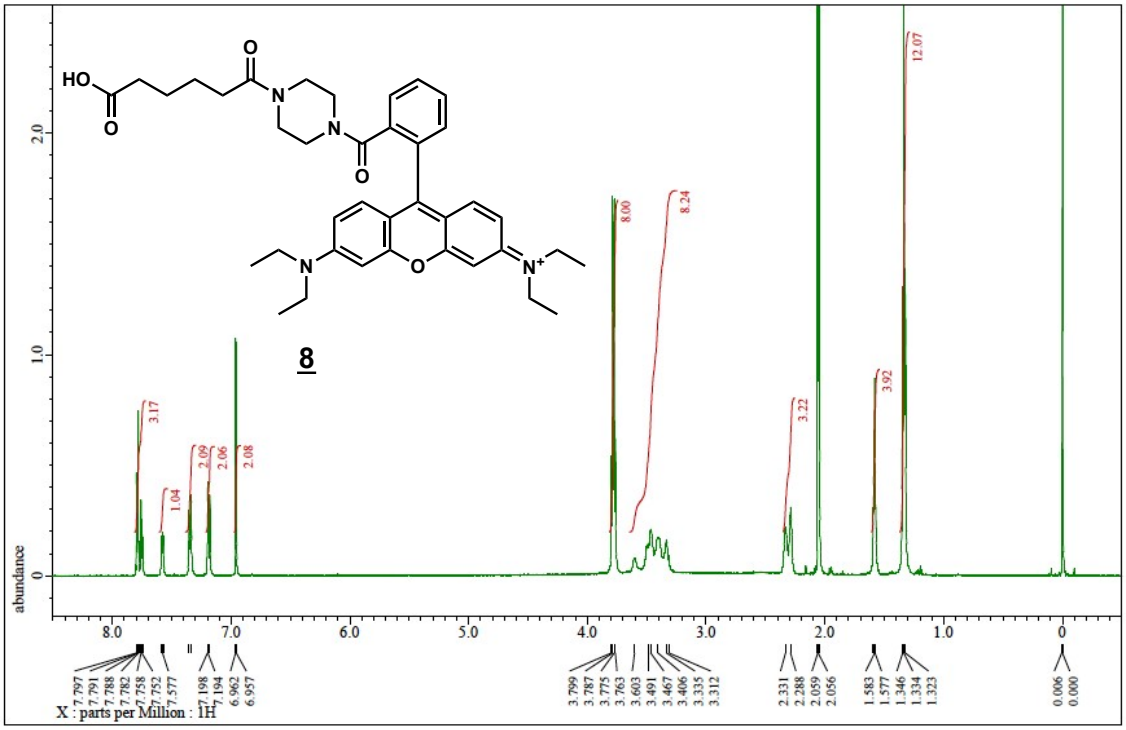

10.539
7.893
7.878
7.672
7.657
7.649
7.634
7.427
7.413
7.397
7.328
7.326
7.313
7.312
7.298
7.297
4.508
4.491
4.479
4.462
4.268
4.266
4.253
4.213
4.200
4.186
3.331
3.168
2.593
2.509
2.505
2.502
2.498
2.495
2.086
1.624
ppm
1.22
2.05
2.09
2.07
2.04
0.89
1.71
1.45
FmocHN
OH
O
NH
N
6

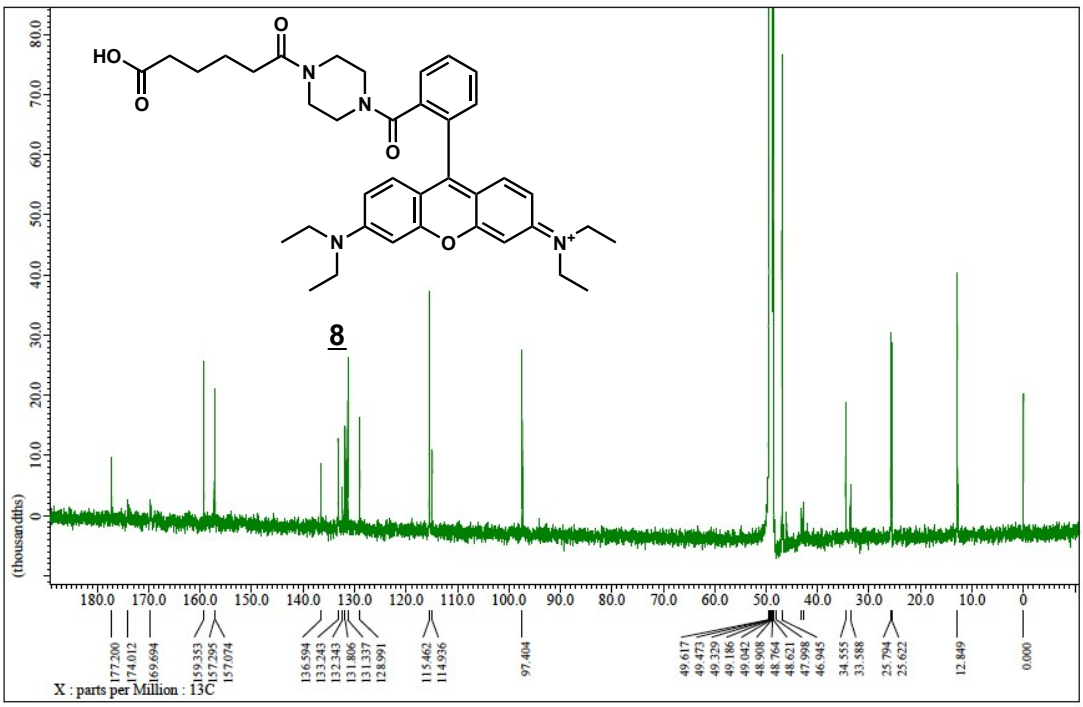

BRUKER
Current Data Parameters
NAME 20211125-2-111-fr11-15
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20211125
Time 18.57
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 55.95
DW 50.000 usec
DE 10.00 usec
TE 298.2 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
SFO1 500.1530886 MHz
NUC1 1H
P1 15.00 usec
PLW1 12.30000019 W
F2 - Processing parameters
SI 65536
SF 500.1500016 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

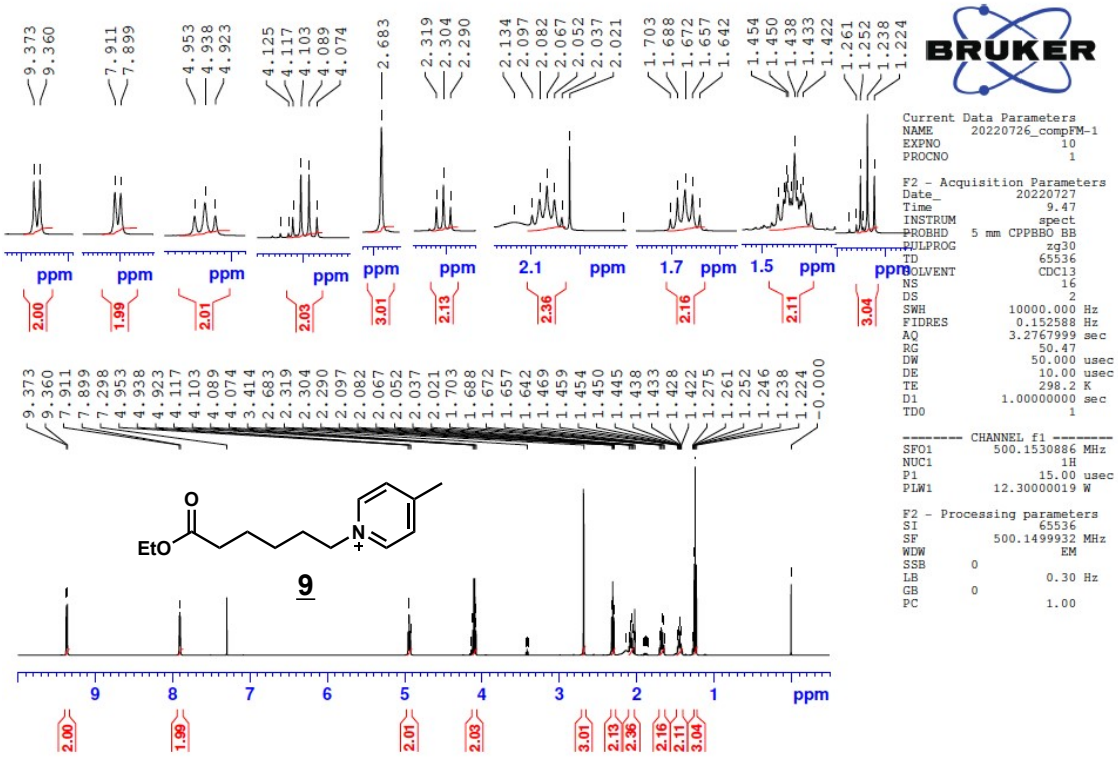

172.46
156.30
144.19
141.15
128.11
127.57
125.72
125.66
120.59
66.23
52.72
47.00
40.49
40.32
40.24
40.15
40.08
39.99
39.82
39.65
39.49
25.86
ppm
FmocHN
OH
O
NH
N
6


BRUKER
Current Data Parameters
NAME 20211203-ffmoc-is2-oh-13c
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20211203
Time 11.19
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.07
DW 16.800 usec
DE 18.00 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 125.7753932 MHz
NUC1 13C
P1 10.00 usec
PLW1 77.00000000 W
CHANNEL f2
SFO2 500.1520006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.30000019 W
PLW12 0.43241999 W
PLW13 0.27675000 W
F2 - Processing parameters
SI 32768
SF 125.7628175 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


FmocHN
N3
3
7
abundance
X : parts per Million : 1H


HO
8
abundance
X : parts per Million : 1H


HO
O
O
N
N
O
O
N
O
N+
8
(thousandths)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
177.200
174.012
169.694
159.353
157.295
157.074
136.594
133.243
132.343
131.806
131.337
128.991
115.462
114.936
97.404
49.617
49.473
49.329
49.186
49.042
48.908
48.764
48.621
47.998
46.945
34.555
33.588
25.794
25.622
12.849
0.000
X : parts per Million : 13C

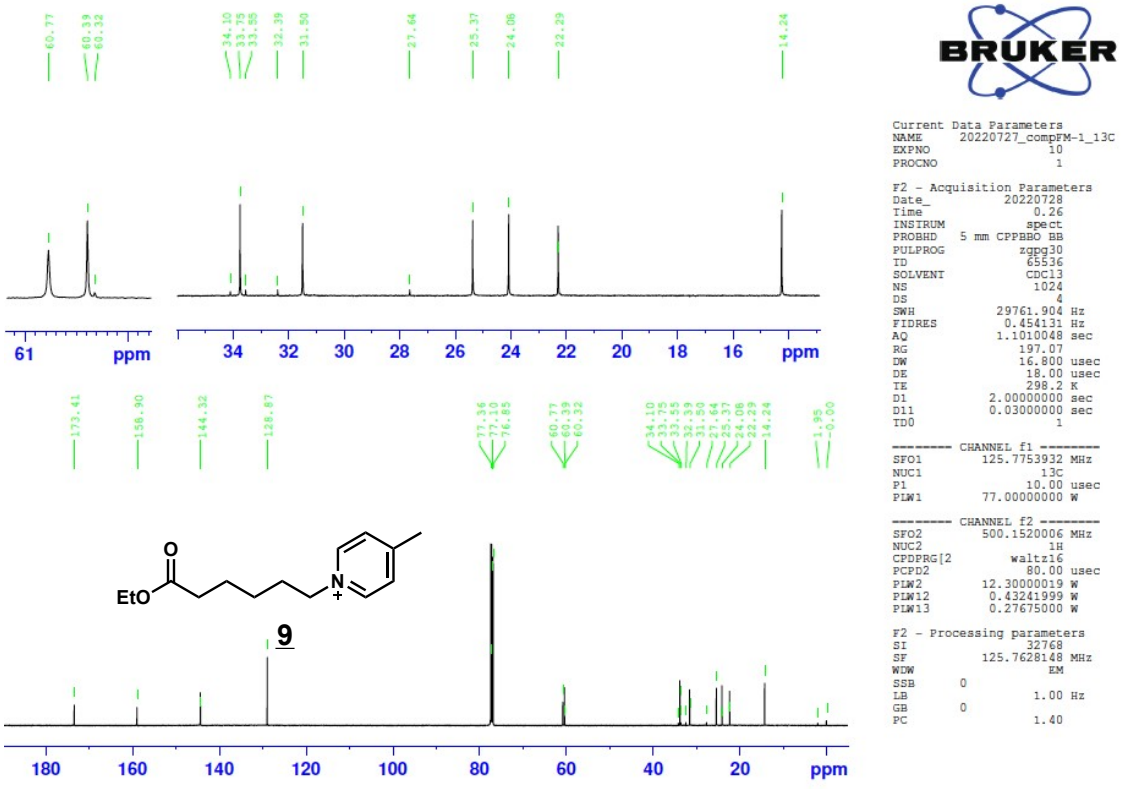



```
Current Data Parameters
NAME      20220726_compFM-1
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20220727
Time                   9.47
INSTRUM               spect
PROBHD   5 mm CPPBBO BB
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH               10000.000 Hz
FIDRES                0.152588 Hz
AQ                3.2767999 sec
RG                     50.47
DW                  50.000 usec
DE                  10.00 usec
TE                    298.2 K
D1                1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1            500.1530886 MHz
NUC1                      1H
P1                     15.00 usec
PLW1             12.30000019 W

F2 - Processing parameters
SI                        65536
SF              500.1499932 MHz
WDW                       EM
SSB          0
LB                     0.30 Hz
GB           0
PC                     1.00
```



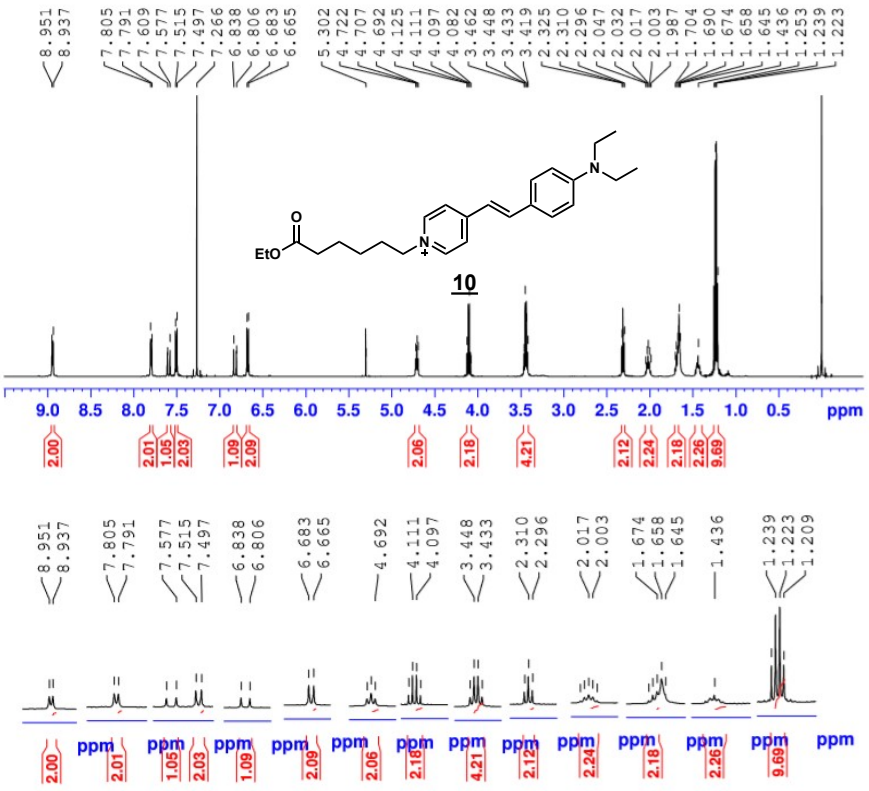

```
Current Data Parameters
NAME      20220727_compFM-1_13C
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20220728
Time                   0.26
INSTRUM               spect
PROBHD   5 mm CPPBBO BB
PULPROG             zgpg30
TD                    65536
SOLVENT               CDCl3
NS                     1024
DS                        4
SWH               29761.904 Hz
FIDRES                0.454131 Hz
AQ                1.1010048 sec
RG                    197.07
DW                  16.800 usec
DE                  18.00 usec
TE                    298.2 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1            125.7753932 MHz
NUC1                      13C
P1                     10.00 usec
PLW1             77.00000000 W

======== CHANNEL f2 ========
SFO2            500.1520006 MHz
NUC2                      1H
CPDPRG[2             waltz16
PCPD2                  80.00 usec
PLW2             12.30000019 W
PLW12             0.43241999 W
PLW13             0.27675000 W

F2 - Processing parameters
SI                        32768
SF              125.7628148 MHz
WDW                       EM
SSB          0
LB                     1.00 Hz
GB           0
PC                     1.40
```

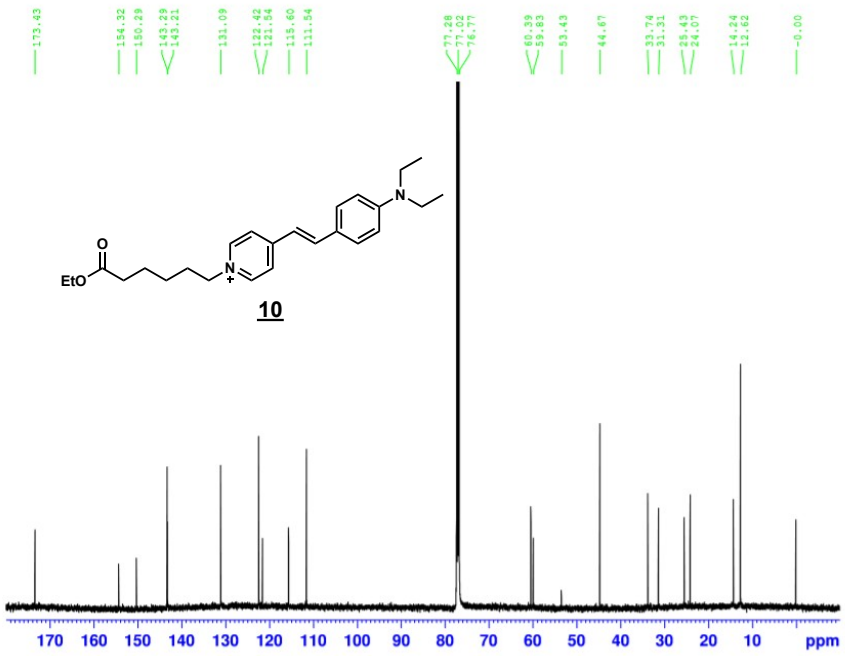


```
Current Data Parameters
NAME          20180405_FME
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20180405
Time                 20.07
INSTRUM              spect
PROBHD   5 mm CPPBBO BB
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH           10000.000 Hz
FIDRES            0.152588 Hz
AQ                3.2767999 sec
RG                   69.94
DW                  50.000 usec
DE                  10.00 usec
TE                  298.1 K
D1             1.00000000 sec
TD0                      1

========== CHANNEL f1 ==========
SFO1          500.1530886 MHz
NUC1                     1H
P1                   15.00 usec
PLW1          12.30000019 W

F2 - Processing parameters
SI                   65536
SF            500.1500086 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```



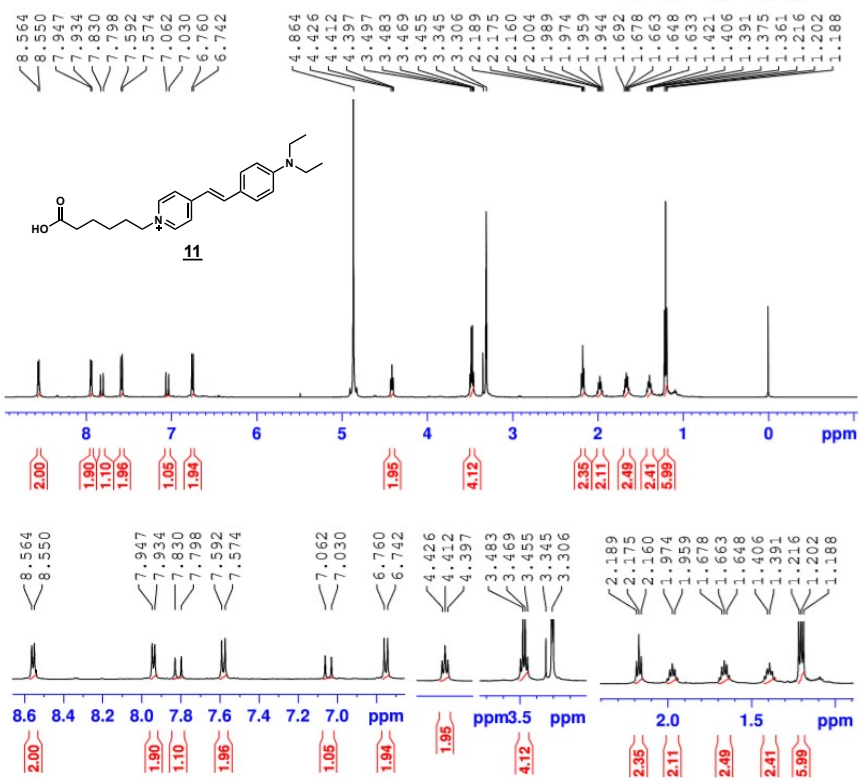
```
Current Data Parameters
NAME      20180405_FME1-13C-clf
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20180406
Time                  1.50
INSTRUM              spect
PROBHD   5 mm CPPBBO BB
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                   2048
DS                      4
SWH           29761.904 Hz
FIDRES            0.454131 Hz
AQ                1.1010048 sec
RG                  197.07
DW                  16.800 usec
DE                  18.00 usec
TE                  298.2 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                      1

========== CHANNEL f1 ==========
SFO1          125.7753932 MHz
NUC1                     13C
P1                   10.00 usec
PLW1          77.00000000 W

========== CHANNEL f2 ==========
SFO2          500.1520006 MHz
NUC2                     1H
CPDPRG[2          waltz16
PCPD2                80.00 usec
PLW2          12.30000019 W
PLW12          0.43241999 W
PLW13          0.27675000 W

F2 - Processing parameters
SI                   32768
SF            125.7628182 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```



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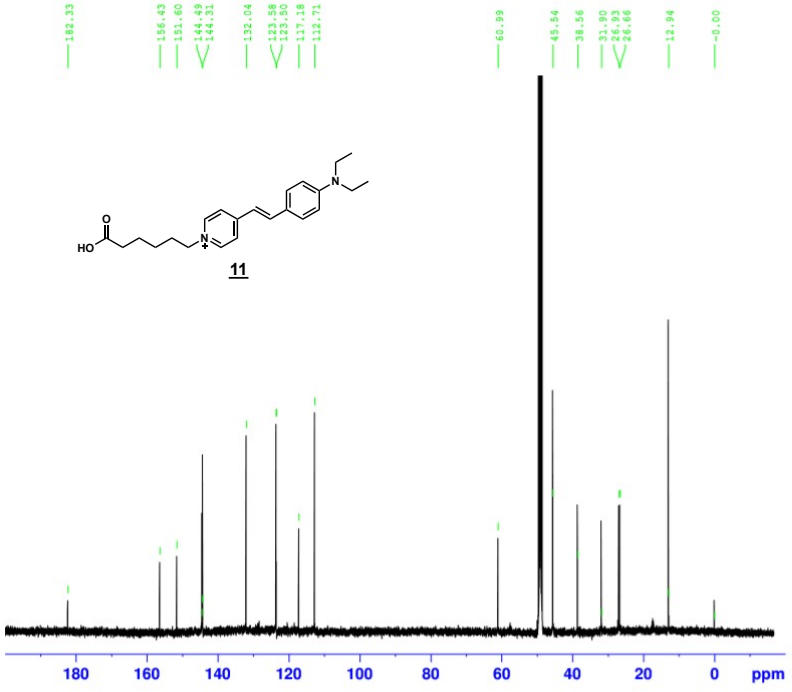
```
Current Data Parameters
NAME        20180515_FME2
EXPNO               1
PROCNO              1

F2 - Acquisition Parameters
Date_          20180515
Time              14.52
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zg30
TD               65536
SOLVENT           MeOD
NS                  16
DS                   2
SWH          10000.000 Hz
FIDRES           0.152588 Hz
AQ            3.2767999 sec
RG                55.95
DW              50.000 usec
DE              10.00 usec
TE                298.2 K
D1           1.00000000 sec
TD0                 1

======== CHANNEL f1 ========
SFO1         500.1530886 MHz
NUC1               1H
P1               15.00 usec
PLW1        12.30000019 W

F2 - Processing parameters
SI               65536
SF          500.1500116 MHz
WDW                EM
SSB         0
LB                0.30 Hz
GB          0
PC                1.00
```



BRUKER

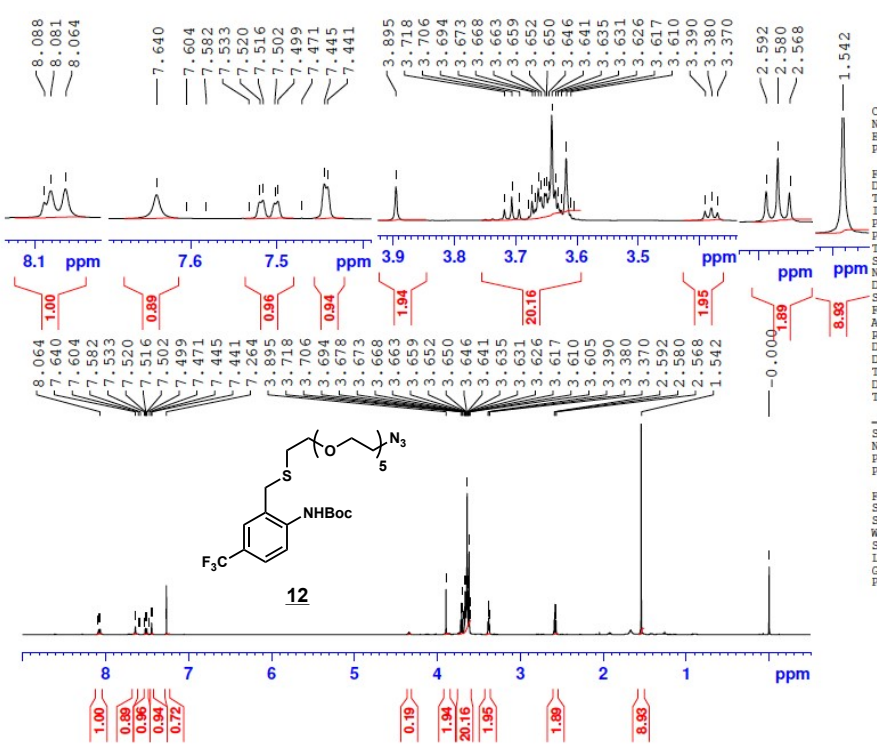
```
Current Data Parameters
NAME      20180515_FME2-13C
EXPNO               1
PROCNO              1

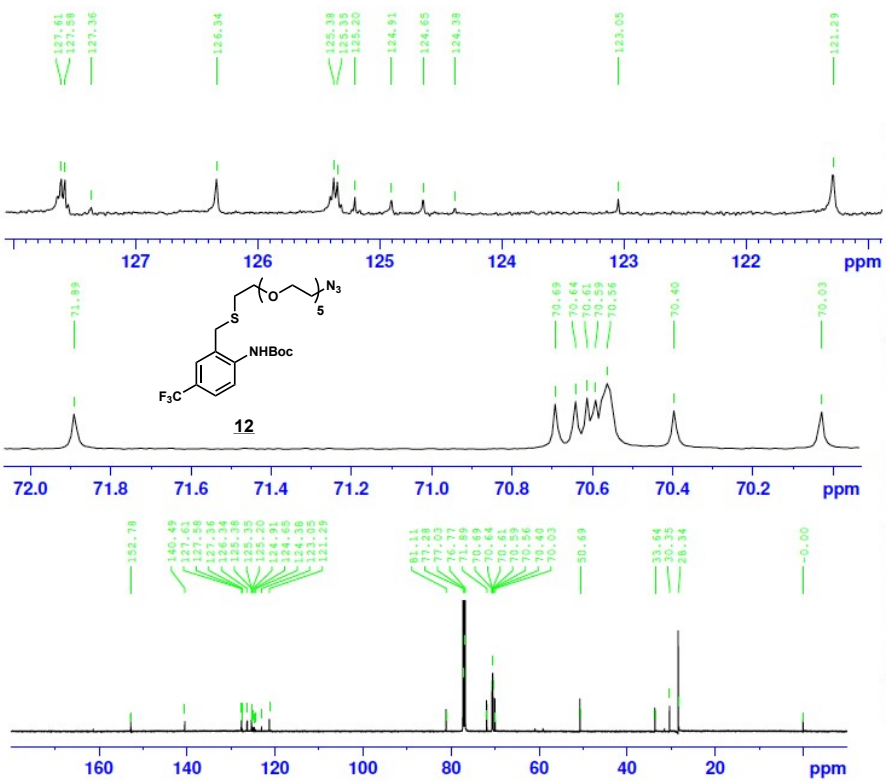
F2 - Acquisition Parameters
Date_          20180515
Time              19.05
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG         zgpg30
TD               65536
SOLVENT           MeOD
NS                1024
DS                   4
SWH          29761.904 Hz
FIDRES           0.454131 Hz
AQ            1.1010048 sec
RG                197.07
DW              16.800 usec
DE              18.00 usec
TE                298.2 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                 1

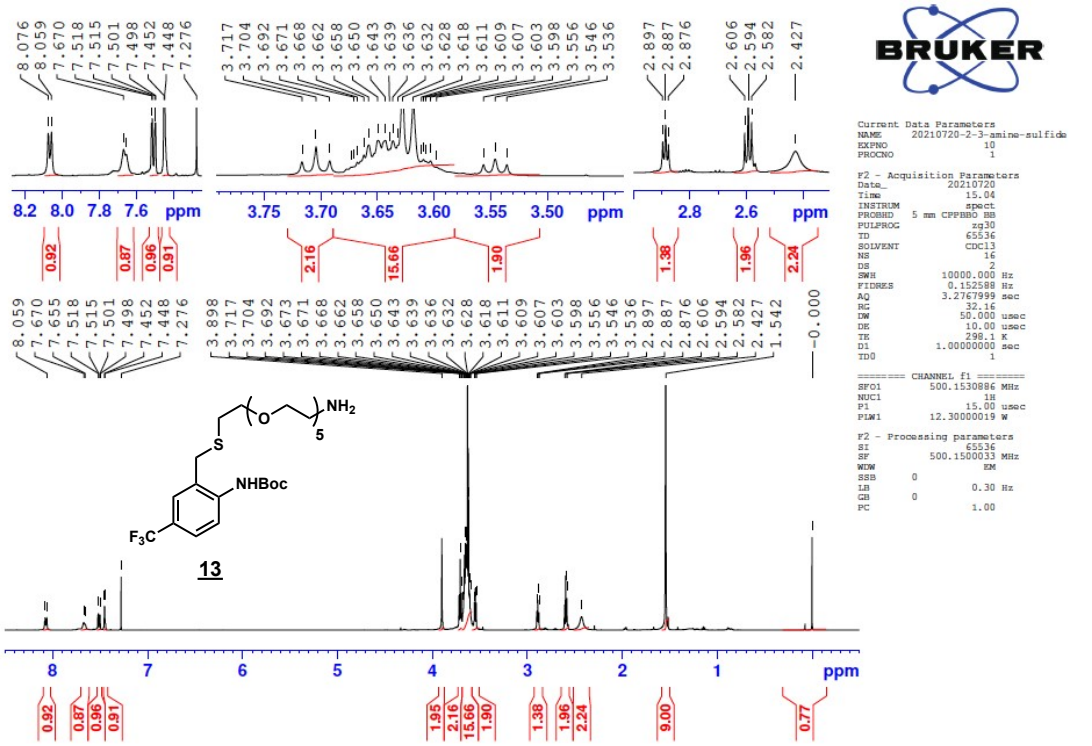
======== CHANNEL f1 ========
SFO1        125.7753932 MHz
NUC1               13C
P1              10.00 usec
PLW1        77.00000000 W

======== CHANNEL f2 ========
SFO2        500.1520006 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2            80.00 usec
PLW2        12.30000019 W
PLW12        0.43241999 W
PLW13        0.27675000 W

F2 - Processing parameters
SI               32768
SF          125.7626368 MHz
WDW                EM
SSB         0
LB                1.00 Hz
GB          0
PC                1.40
```


12


12



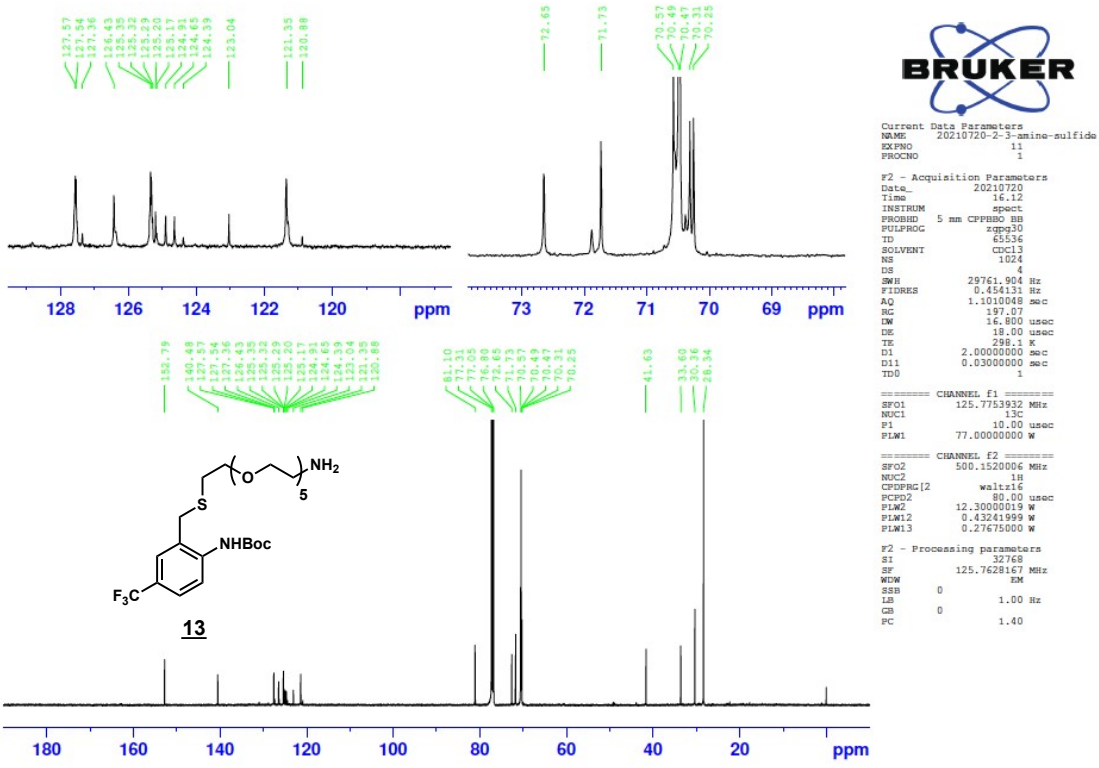
BRUKER

```
Current Data Parameters
NAME      20210720-2-3-amine-sulfide
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_              20210720
Time                 15.04
INSTRUM              spect
PROBHD    5 mm CPPBBO BB
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH             10000.000 Hz
FIDRES              0.152588 Hz
AQ                3.2767999 sec
RG                    32.16
DW                  50.000 usec
DE                  10.00 usec
TE                  298.1 K
D1                1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1            500.1530886 MHz
NUC1                    1H
P1                   15.00 usec
PLW1            12.30000019 W

F2 - Processing parameters
SI                    65536
SF              500.1500033 MHz
WDW                     EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```



BRUKER

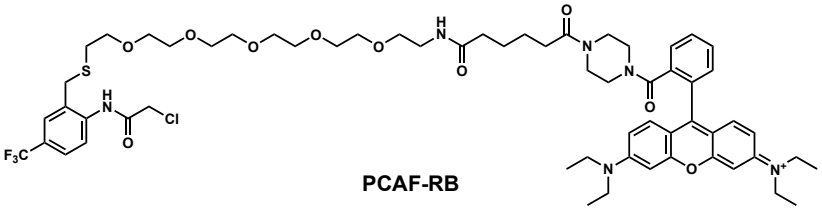
```
Current Data Parameters
NAME      20210720-2-3-amine-sulfide
EXPNO                11
PROCNO                1

F2 - Acquisition Parameters
Date_              20210720
Time                 16.12
INSTRUM              spect
PROBHD    5 mm CPPBBO BB
PULPROG           zgpg30
TD                  65536
SOLVENT             CDCl3
NS                   1024
DS                      4
SWH             29761.904 Hz
FIDRES              0.454131 Hz
AQ                1.1010048 sec
RG                    197.07
DW                  16.800 usec
DE                  18.00 usec
TE                  298.1 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                    1

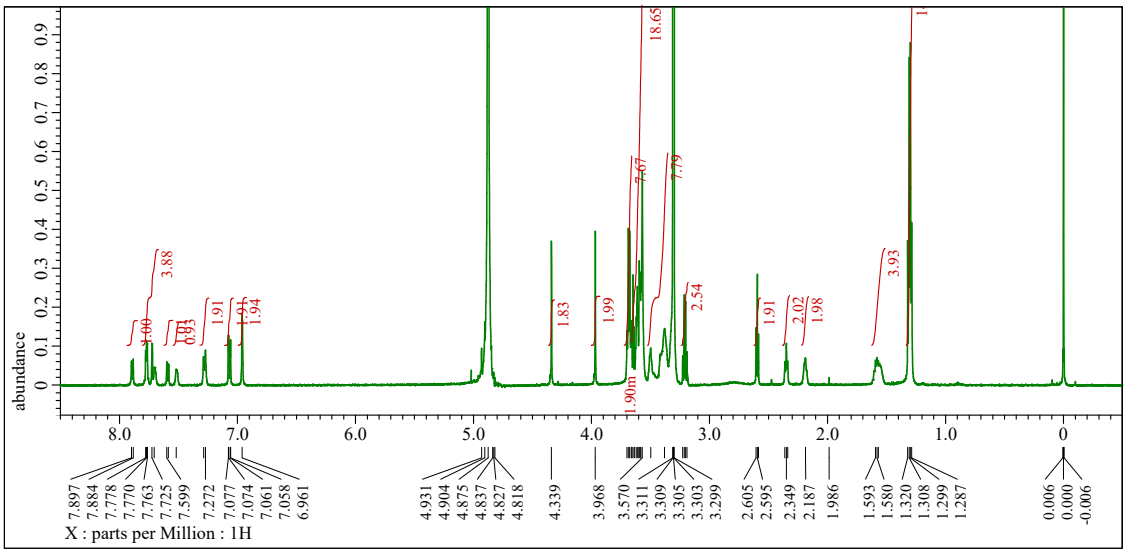
======== CHANNEL f1 ========
SFO1            125.7753932 MHz
NUC1                    13C
P1                   10.00 usec
PLW1            77.00000000 W

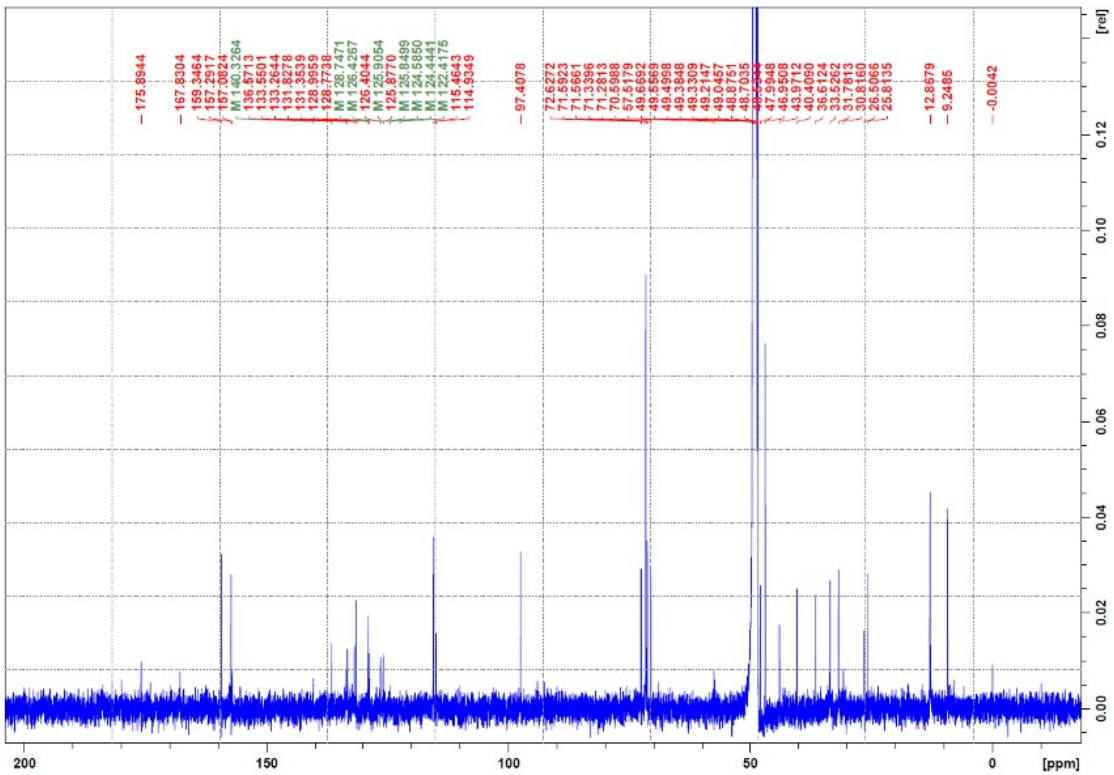
======== CHANNEL f2 ========
SFO2            500.1520006 MHz
NUC2                    1H
CPDPRG[2             waltz16
PCPD2                80.00 usec
PLW2            12.30000019 W
PLW12            0.43241999 W
PLW13            0.27675000 W

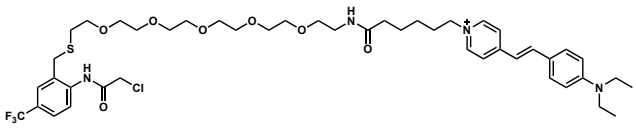
F2 - Processing parameters
SI                    32768
SF              125.7628167 MHz
WDW                     EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```



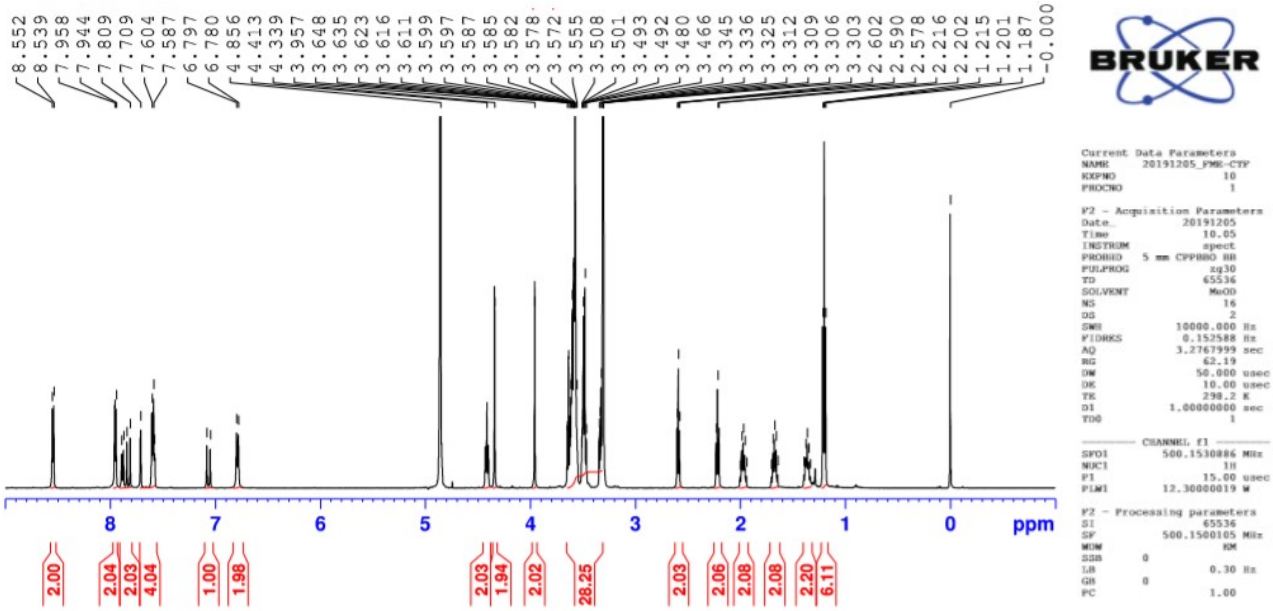
PCAF-RB



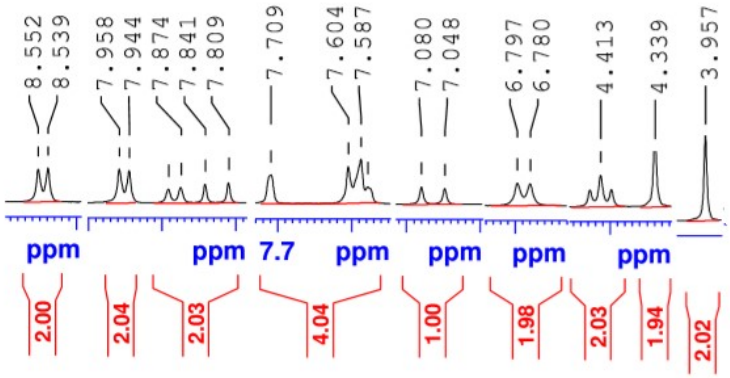


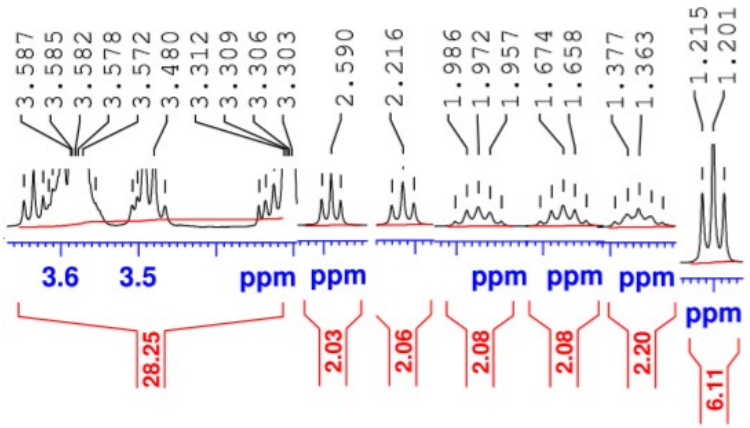


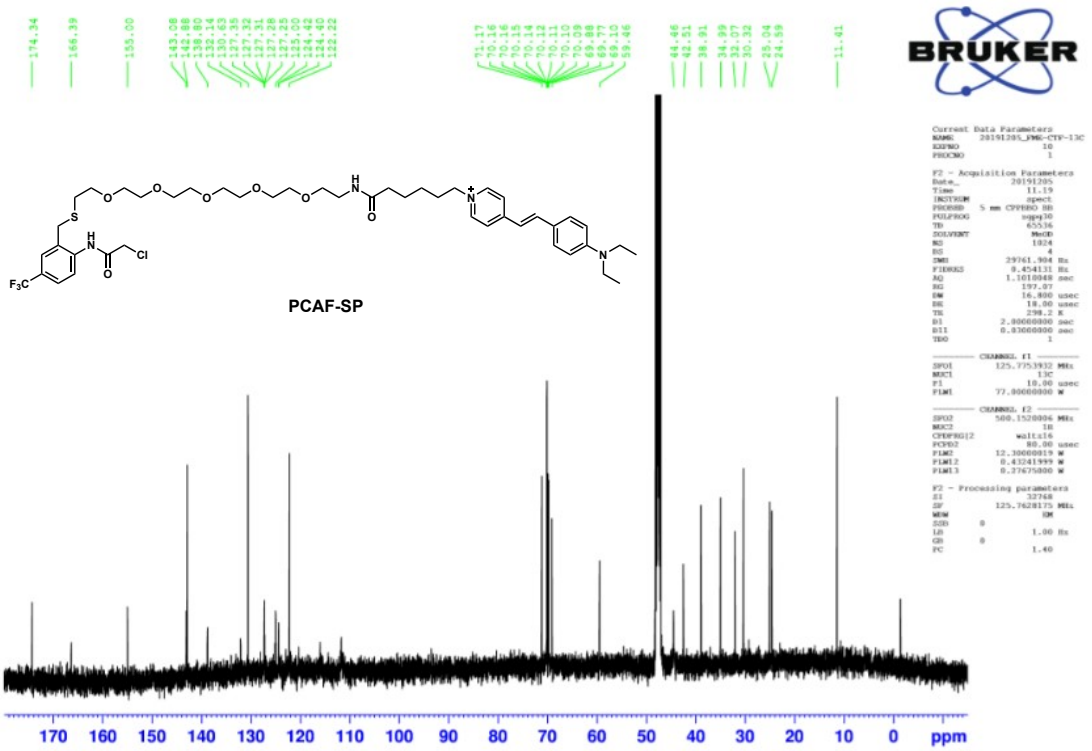
PCAF-SP

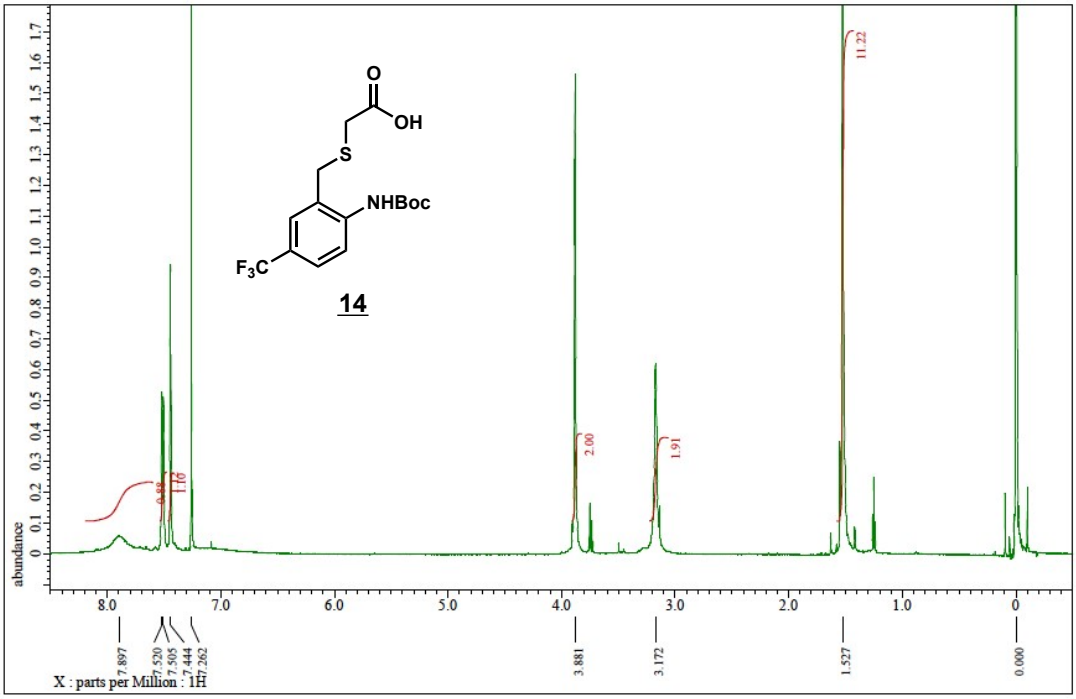

8.552
8.539
7.958
7.944
7.809
7.709
7.604
7.587
6.797
6.780
4.856
4.413
4.339
3.957
3.648
3.635
3.623
3.616
3.611
3.599
3.597
3.587
3.585
3.582
3.578
3.572
3.555
3.508
3.501
3.493
3.492
3.480
3.466
3.345
3.336
3.325
3.312
3.309
3.306
3.303
2.602
2.590
2.578
2.216
2.202
1.215
1.201
1.187
0.000
8
7
6
5
4
3
2
1
0
ppm
2.00
2.04
2.03
4.04
1.00
1.98
2.03
1.94
2.02
28.25
2.03
2.06
2.08
2.08
2.20
6.11

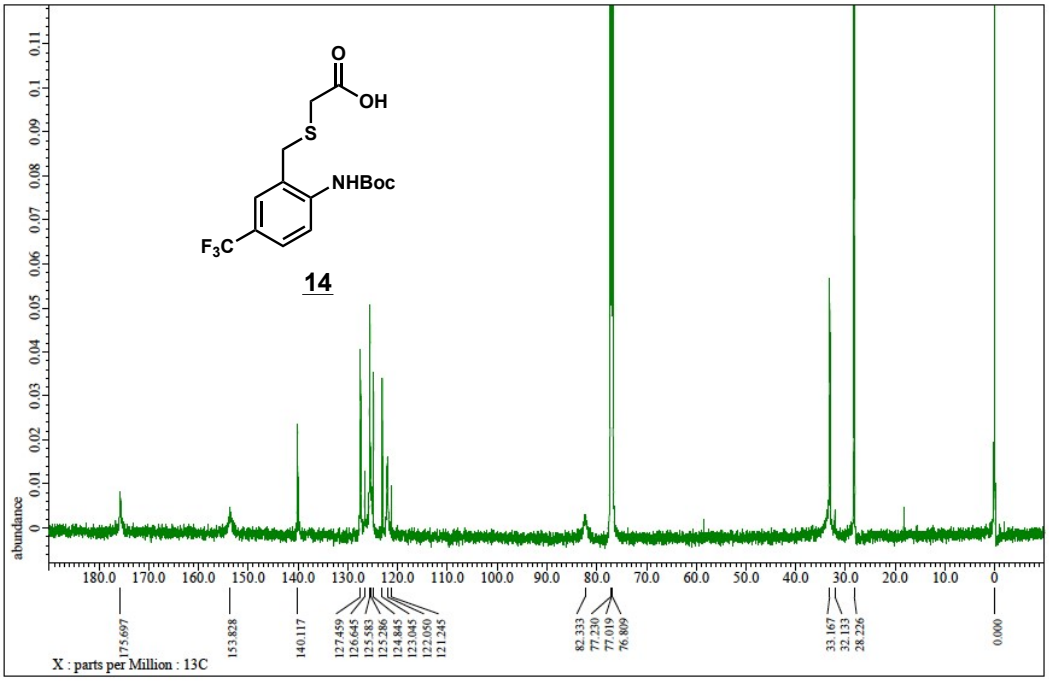
Current Data Parameters
NAME 20191205_PME-CTF
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191205
Time 10.05
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 62.19
DW 50.000 usec
DE 10.00 usec
TE 298.2 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
SFO1 500.1530886 MHz
NUC1 1H
P1 15.00 usec
PLW1 12.30000019 W
F2 - Processing parameters
SI 65536
SF 500.1500105 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


8.552
8.539
7.958
7.944
7.874
7.841
7.809
7.709
7.604
7.587
7.080
7.048
6.797
6.780
4.413
4.339
3.957
ppm
ppm
7.7
ppm
ppm
ppm
ppm
2.00
2.04
2.03
4.04
1.00
1.98
2.03
1.94
2.02

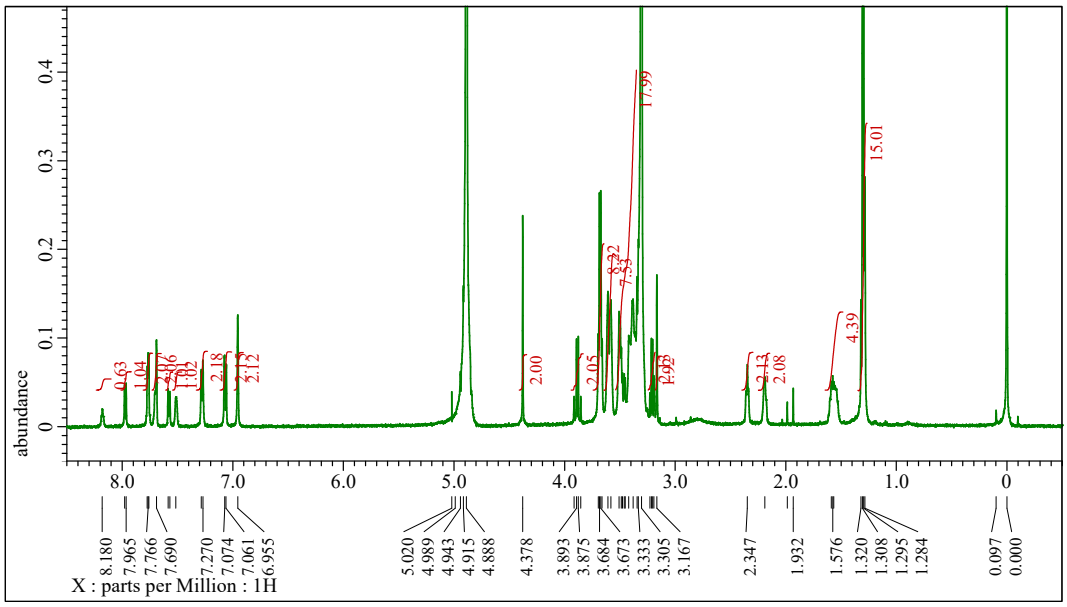

3.587
3.585
3.582
3.578
3.572
3.480
3.312
3.309
3.306
3.303
2.590
2.216
1.986
1.972
1.957
1.674
1.658
1.377
1.363
1.215
1.201
3.6
3.5
ppm
ppm
ppm
ppm
ppm
ppm
28.25
2.03
2.06
2.08
2.08
2.20
6.11

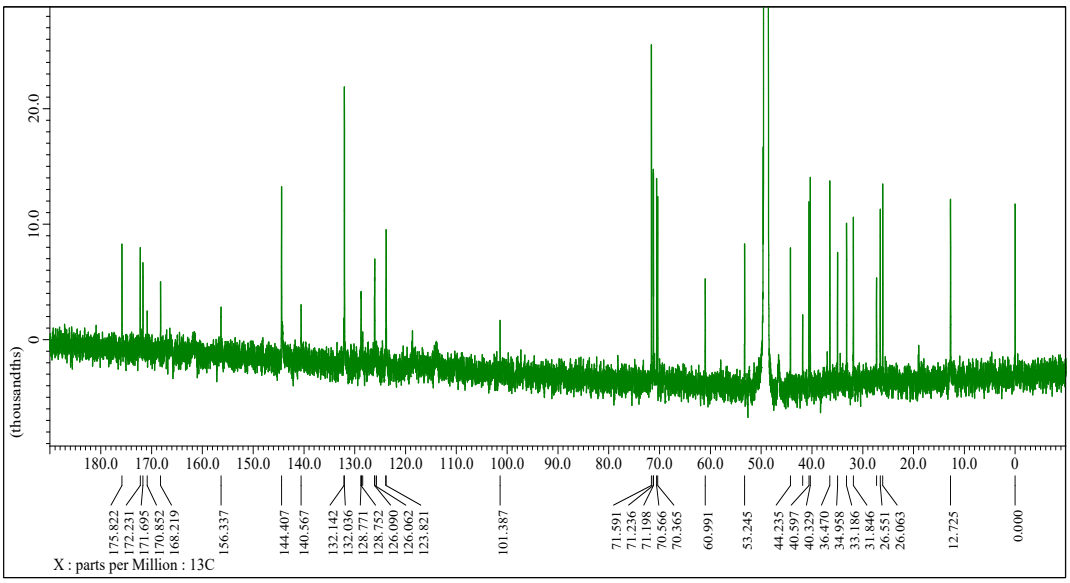

PCAF-SP
ppm

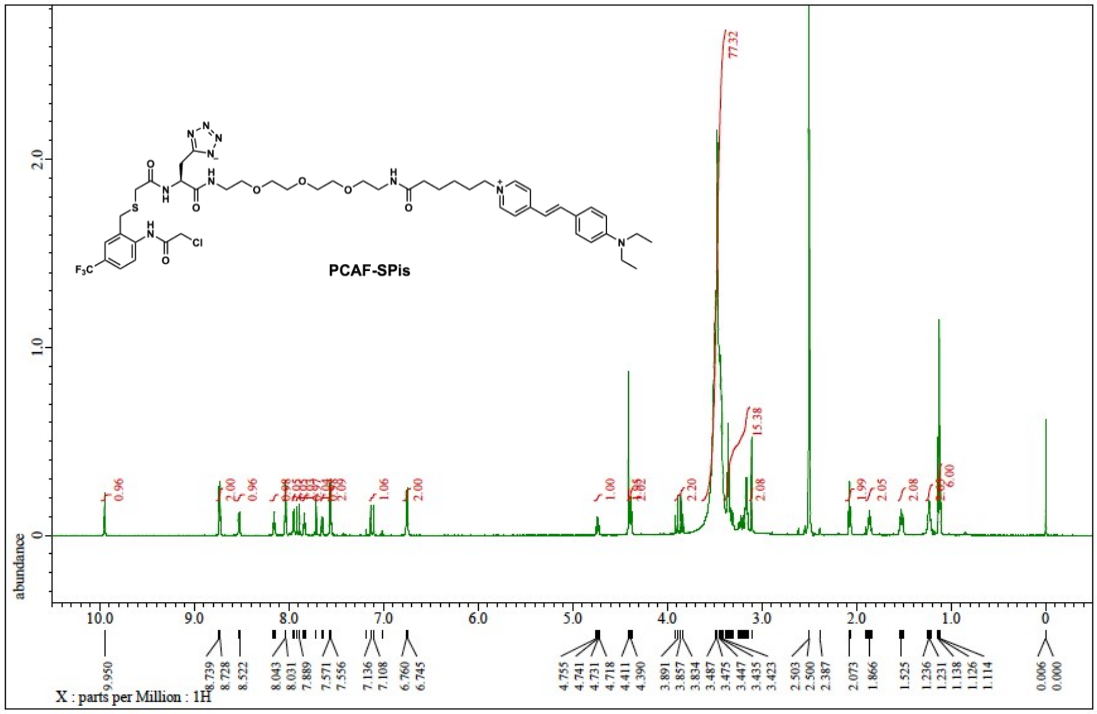

O
OH
S
NHBoc
F3C
14
abundance
11.22
2.00
1.91
1.16
0.89
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.897
7.520
7.505
7.444
7.262
3.881
3.172
1.527
0.000
X : parts per Million : 1H

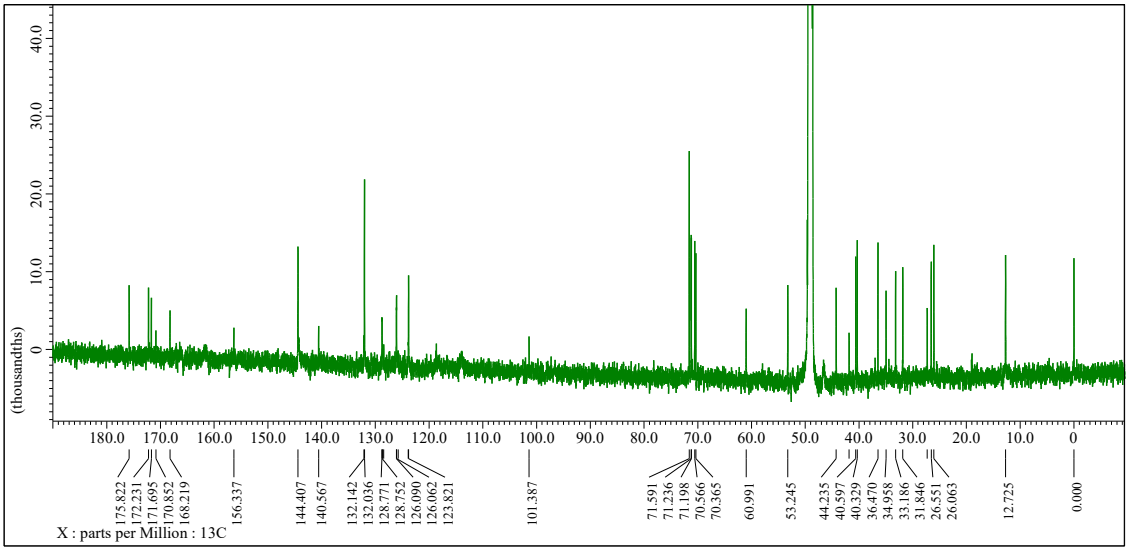

O
OH
S
NHBoc
F3C
14
abundance
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
175.697
153.828
140.117
127.459
126.645
125.583
125.286
124.845
123.045
122.050
121.245
82.333
77.230
77.019
76.809
33.167
32.133
28.226
0.000
X : parts per Million : 13C

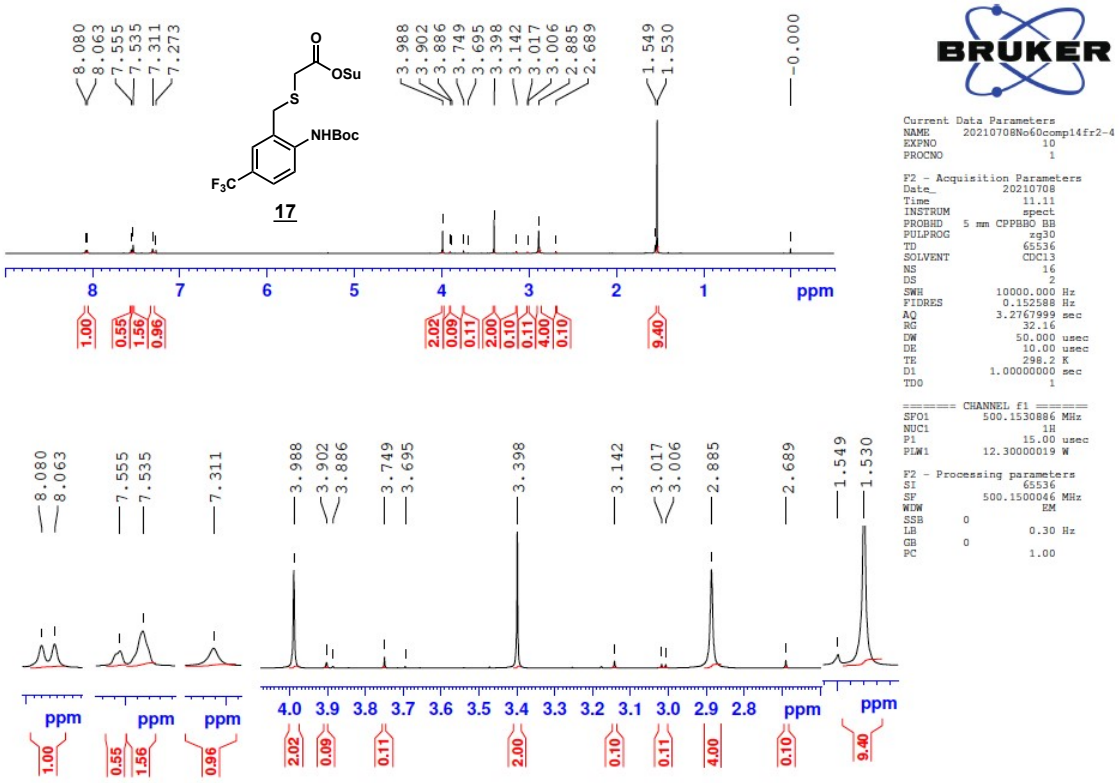
PCAF-RBis


abundance
X : parts per Million : 1H


(thousandths)
X : parts per Million : 13C


PCAF-SPis
abundance
X : parts per Million : 1H


(thousandths)
X : parts per Million : 13C



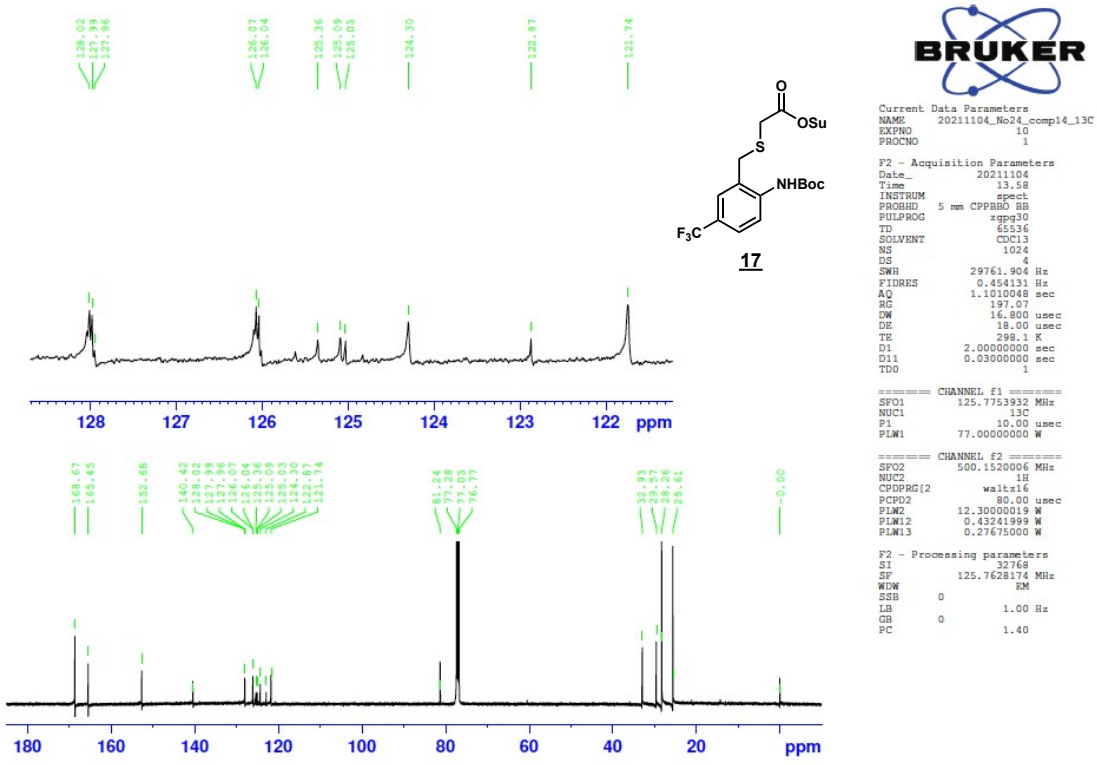
BRUKER

```
Current Data Parameters
NAME      20210708No60comp14fr2-4
EXPNO                          10
PROCNO                          1

F2 - Acquisition Parameters
Date_                    20210708
Time                        11.11
INSTRUM                     spect
PROBHD    5 mm CPPBBO BB
PULPROG                     zg30
TD                         65536
SOLVENT                    CDCl3
NS                             16
DS                              2
SWH                    10000.000 Hz
FIDRES                     0.152588 Hz
AQ                     3.2767999 sec
RG                          32.16
DW                         50.000 usec
DE                         10.00 usec
TE                         298.2 K
D1                    1.00000000 sec
TD0                             1

======== CHANNEL f1 ========
SFO1                 500.1530886 MHz
NUC1                           1H
P1                         15.00 usec
PLW1                12.30000019 W

F2 - Processing parameters
SI                         65536
SF                   500.1500046 MHz
WDW                            EM
SSB                 0
LB                         0.30 Hz
GB                  0
PC                         1.00
```



BRUKER

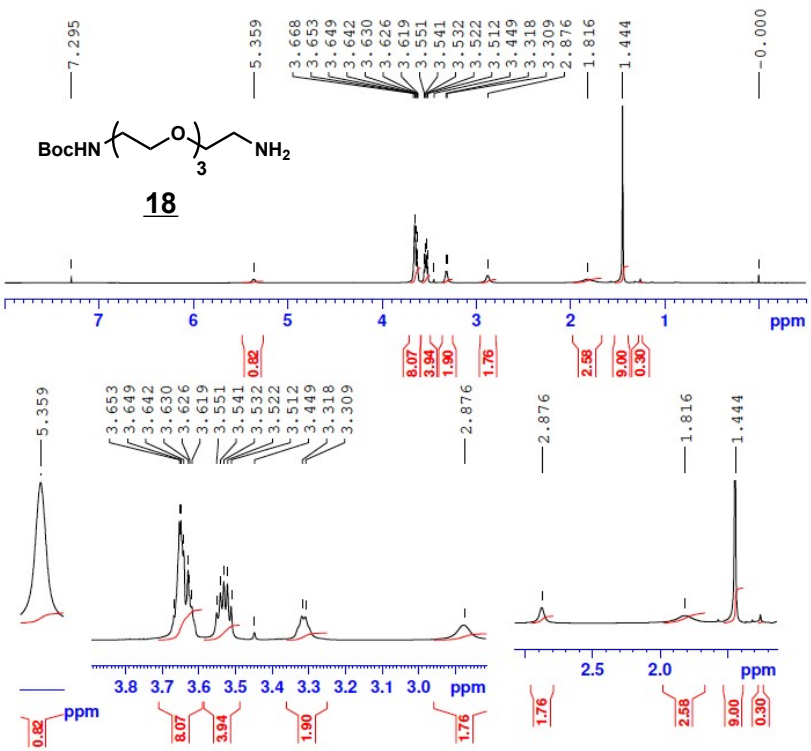
```
Current Data Parameters
NAME      20211104_No24_comp14_13C
EXPNO                          10
PROCNO                          1

F2 - Acquisition Parameters
Date_                    20211104
Time                        13.58
INSTRUM                     spect
PROBHD    5 mm CPPBBO BB
PULPROG                   zgpg30
TD                         65536
SOLVENT                    CDCl3
NS                           1024
DS                              4
SWH                    29761.904 Hz
FIDRES                     0.454131 Hz
AQ                     1.1010048 sec
RG                          197.07
DW                         16.800 usec
DE                         18.00 usec
TE                         298.1 K
D1                     2.00000000 sec
D11                    0.03000000 sec
TD0                             1

======== CHANNEL f1 ========
SFO1                 125.7753932 MHz
NUC1                           13C
P1                         10.00 usec
PLW1                77.00000000 W

======== CHANNEL f2 ========
SFO2                 500.1520006 MHz
NUC2                           1H
CPDPRG[2                  waltz16
PCPD2                      80.00 usec
PLW2                12.30000019 W
PLW12                0.43241999 W
PLW13                0.27675000 W

F2 - Processing parameters
SI                         32768
SF                   125.7628174 MHz
WDW                            EM
SSB                 0
LB                         1.00 Hz
GB                  0
PC                         1.40
```

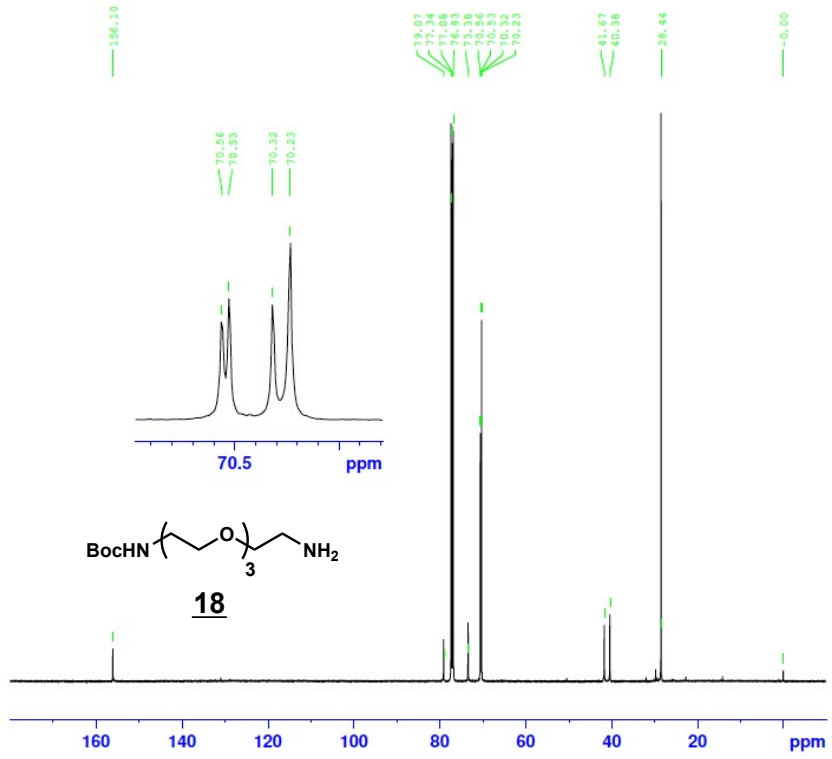


```
Current Data Parameters
NAME        20210911No93
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210911
Time              13.01
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  16
DS                   2
SWH          10000.000 Hz
FIDRES           0.152588 Hz
AQ               3.2767999 sec
RG                  32.16
DW                50.000 usec
DE                10.00 usec
TE                298.2 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         500.1530886 MHz
NUC1                   1H
P1                  15.00 usec
PLW1         12.30000019 W

F2 - Processing parameters
SI                  65536
SF           500.1499937 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



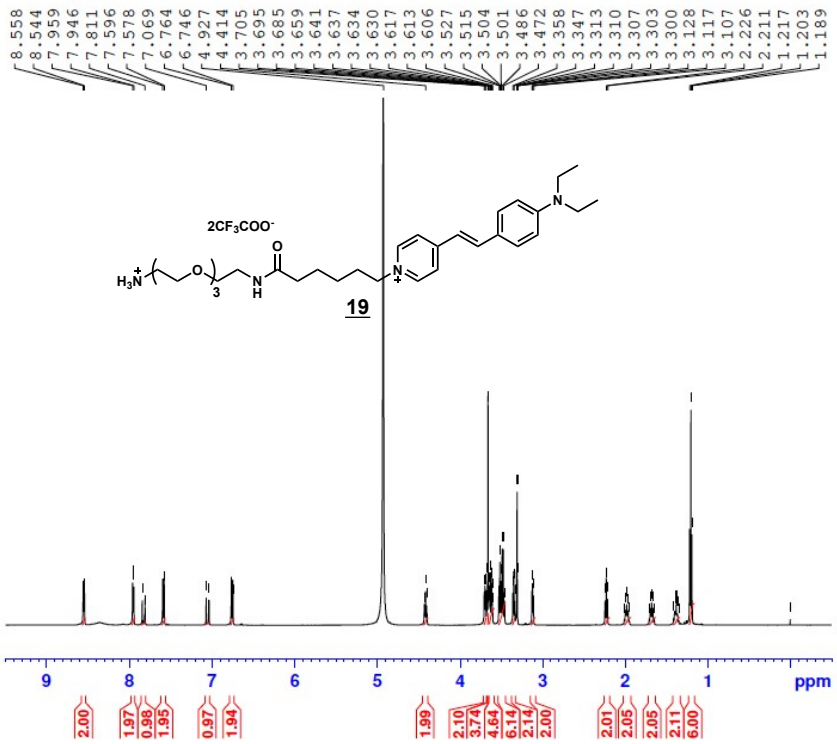
```
Current Data Parameters
NAME    20210911_No93_Boc-linker_13C
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210911
Time              14.06
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG         zgpg30
TD               65536
SOLVENT          CDCl3
NS                1024
DS                   4
SWH          29761.904 Hz
FIDRES           0.454131 Hz
AQ               1.1010048 sec
RG                 197.07
DW                16.800 usec
DE                18.00 usec
TE                298.2 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         125.7753932 MHz
NUC1                  13C
P1                 10.00 usec
PLW1         77.00000000 W

======== CHANNEL f2 ========
SFO2         500.1520006 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2         12.30000019 W
PLW12         0.43241999 W
PLW13         0.27675000 W

F2 - Processing parameters
SI                  32768
SF           125.7628153 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

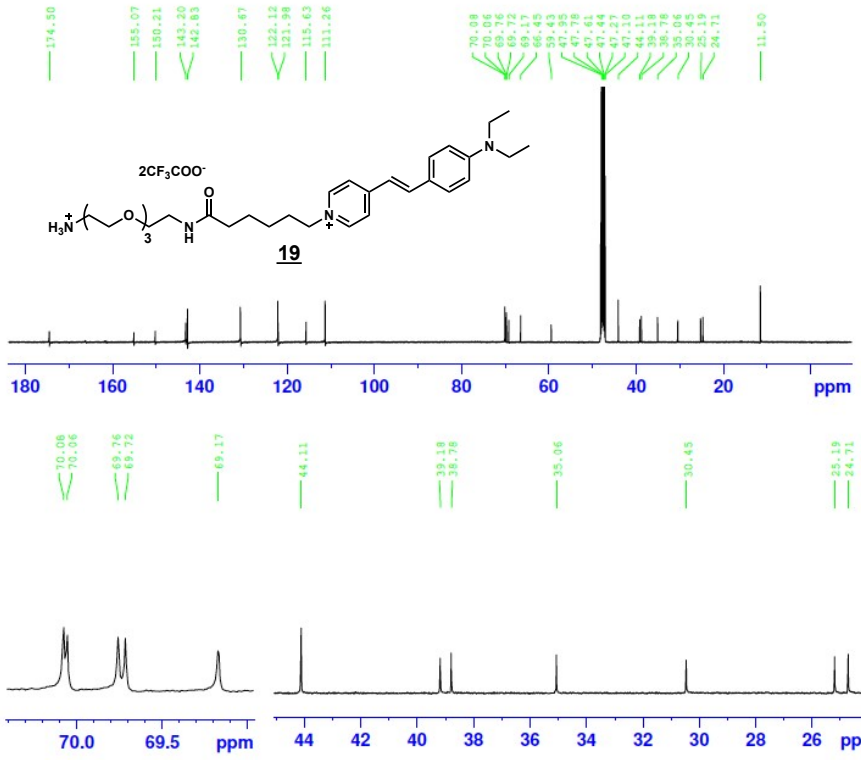



```
Current Data Parameters
NAME      20210923_No101_comp16
EXPNO                        10
PROCNO                        1

F2 - Acquisition Parameters
Date_              20210923
Time                  12.40
INSTRUM                spect
PROBHD    5 mm CPPBBO BB
PULPROG                zg30
TD                    65536
SOLVENT                MeOD
NS                       16
DS                        2
SWH               10000.000 Hz
FIDRES                0.152588 Hz
AQ                3.2767999 sec
RG                       32.16
DW                    50.000 usec
DE                    10.00 usec
TE                    291.2 K
D1                1.00000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1           500.1530886 MHz
NUC1                       1H
P1                    15.00 usec
PLW1           12.30000019 W

F2 - Processing parameters
SI                        65536
SF             500.1500112 MHz
WDW                          EM
SSB          0
LB                     0.30 Hz
GB           0
PC                     1.00
```



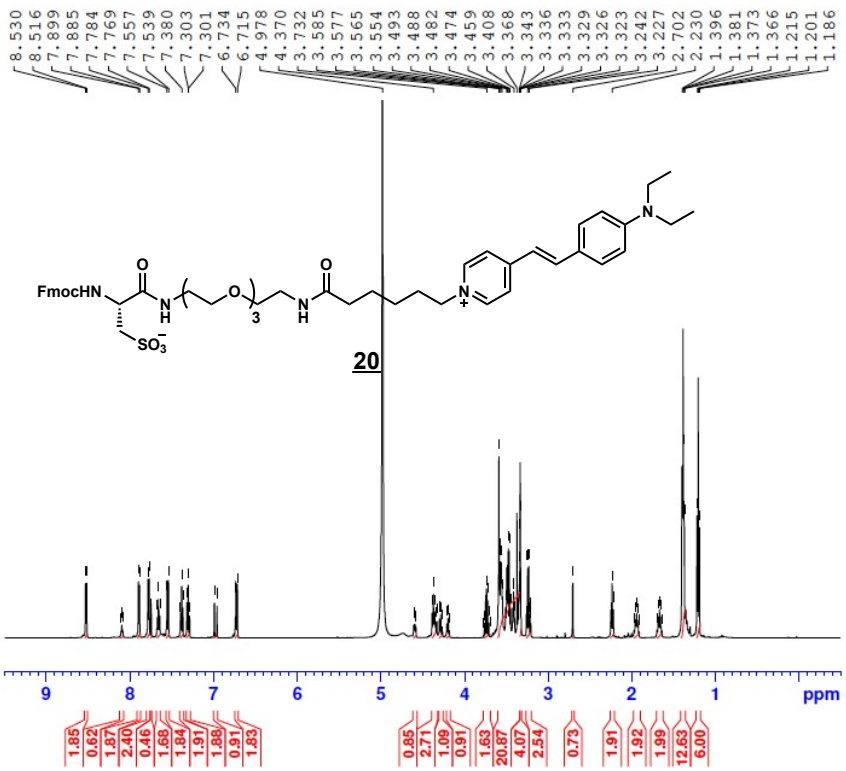

```
Current Data Parameters
NAME      20210923_No101_comp16_13
EXPNO                        10
PROCNO                        1

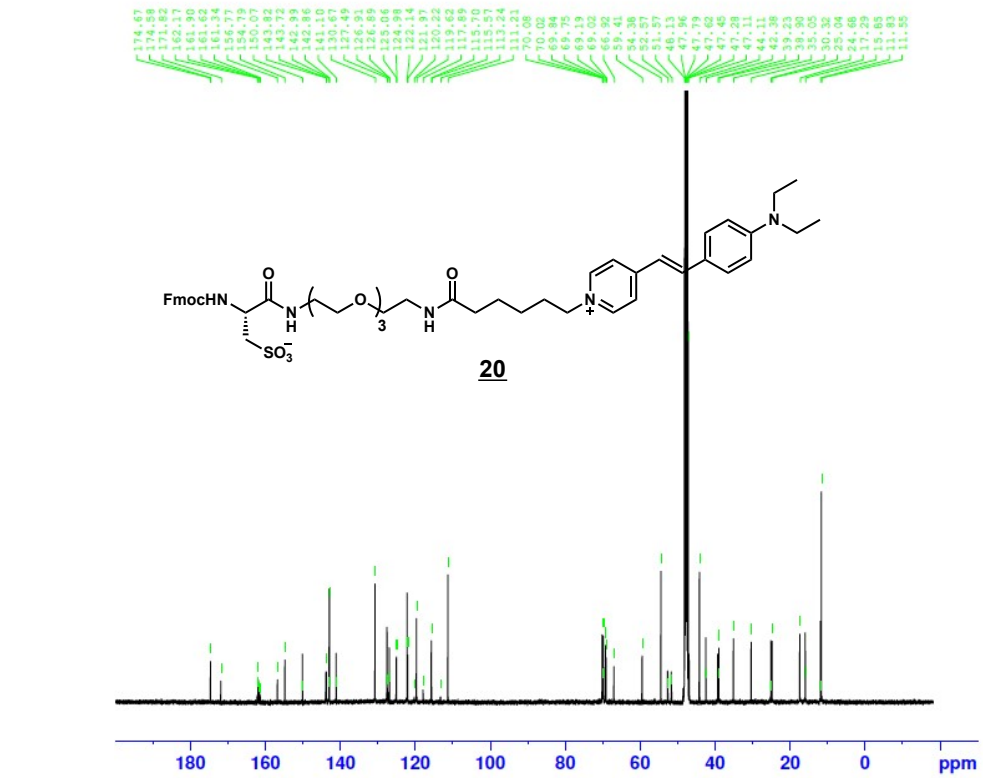
F2 - Acquisition Parameters
Date_              20210923
Time                  14.06
INSTRUM                spect
PROBHD    5 mm CPPBBO BB
PULPROG             zgpg30
TD                    65536
SOLVENT                MeOD
NS                     1024
DS                        4
SWH               29761.904 Hz
FIDRES                0.454131 Hz
AQ                1.1010048 sec
RG                    197.07
DW                    16.800 usec
DE                    18.00 usec
TE                    292.6 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1           125.7753932 MHz
NUC1                       13C
P1                    10.00 usec
PLW1           77.00000000 W

======== CHANNEL f2 ========
SFO2           500.1520006 MHz
NUC2                       1H
CPDPRG[2             waltz16
PCPD2                 80.00 usec
PLW2           12.30000019 W
PLW12           0.43241999 W
PLW13           0.27675000 W

F2 - Processing parameters
SI                        32768
SF             125.7628175 MHz
WDW                          EM
SSB          0
LB                     1.00 Hz
GB           0
PC                     1.40
```






```
Current Data Parameters
NAME      20210927_No110_compl7_fr11-13
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_              20210927
Time                 15.17
INSTRUM              spect
PROBHD    5 mm CPPBBO BB
PULPROG              zg30
TD                  65536
SOLVENT              MeOD
NS                     16
DS                      2
SWH              10000.000 Hz
FIDRES               0.152588 Hz
AQ                3.2767999 sec
RG                    32.16
DW                 50.000 usec
DE                 10.00 usec
TE                   288.5 K
D1                1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1            500.1530886 MHz
NUC1                   1H
P1                  15.00 usec
PLW1           12.30000019 W

F2 - Processing parameters
SI                    65536
SF              500.1500000 MHz
WDW                    EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



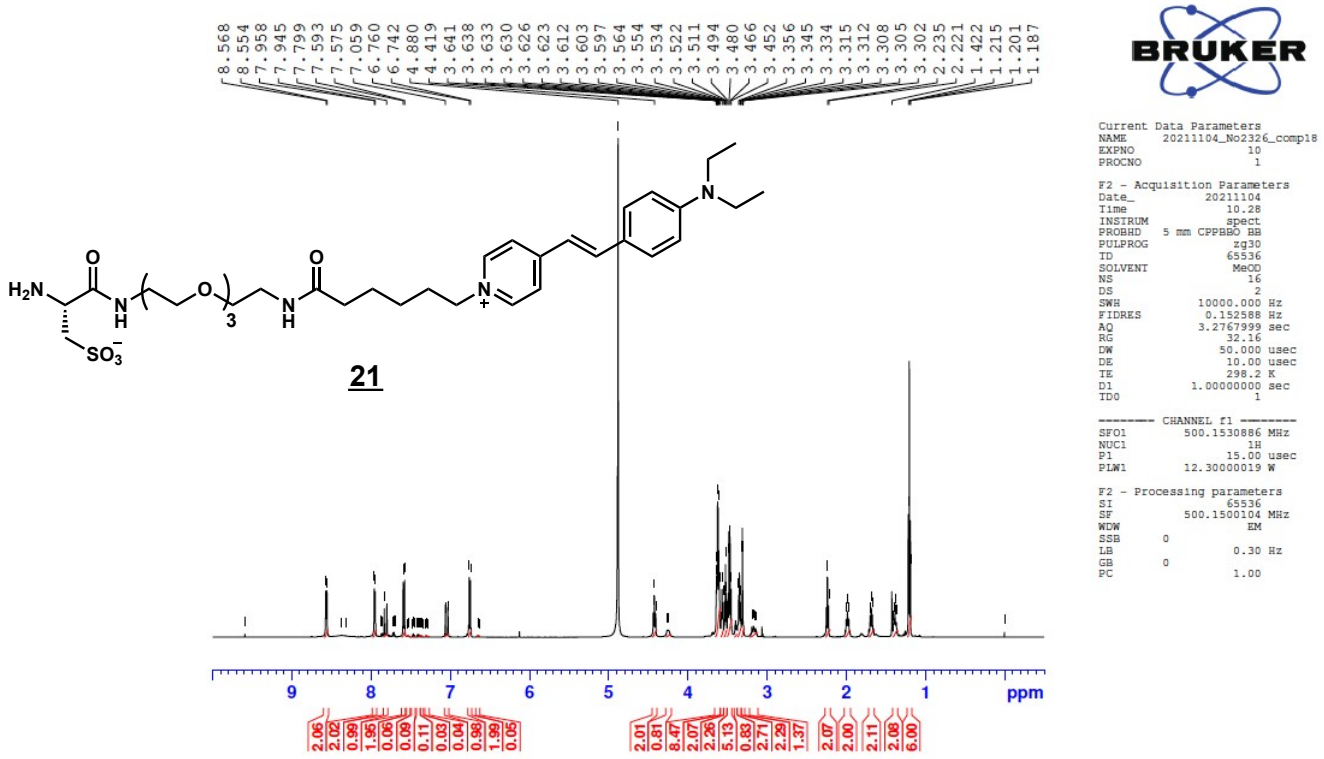
```
Current Data Parameters
NAME      20210927_No110_compl7_fr11-13_13C
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_              20210927
Time                 16.51
INSTRUM              spect
PROBHD    5 mm CPPBBO BB
PULPROG           zgpg30
TD                  65536
SOLVENT              MeOD
NS                   1024
DS                      4
SWH              29761.904 Hz
FIDRES               0.454131 Hz
AQ                1.1010048 sec
RG                   197.07
DW                 16.800 usec
DE                 18.00 usec
TE                   288.2 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1            125.7753932 MHz
NUC1                   13C
P1                  10.00 usec
PLW1           77.00000000 W

======== CHANNEL f2 ========
SFO2            500.1520006 MHz
NUC2                   1H
CPDPRG[2           waltz16
PCPD2               80.00 usec
PLW2           12.30000019 W
PLW12           0.43241999 W
PLW13           0.27675000 W

F2 - Processing parameters
SI                    32768
SF              125.7628175 MHz
WDW                    EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```



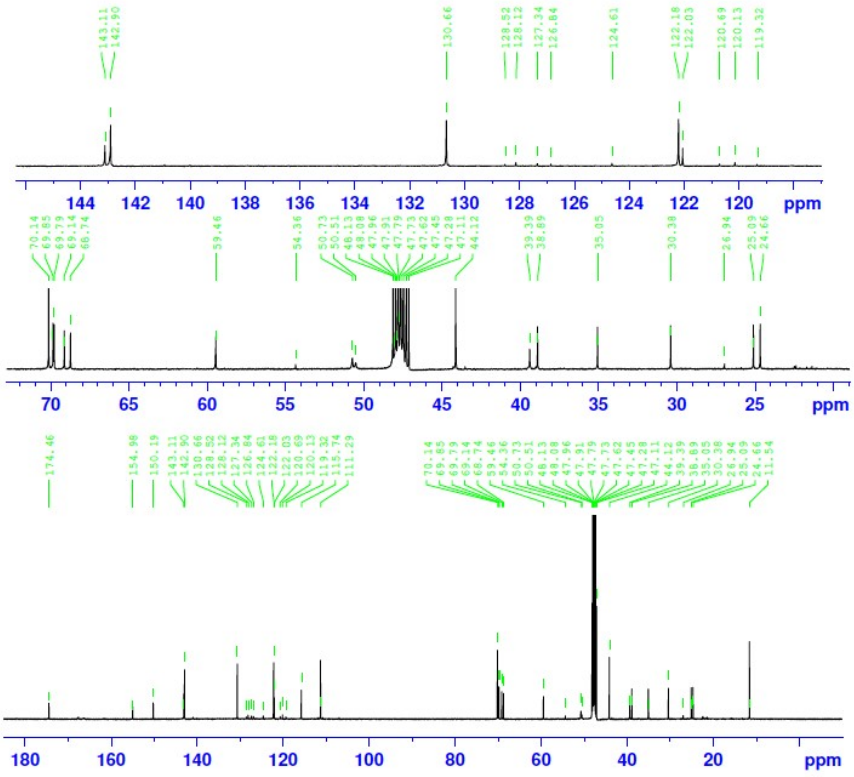
BRUKER

```
Current Data Parameters
NAME      20211104_No2326_comp18
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_              20211104
Time                 10.28
INSTRUM              spect
PROBHD    5 mm CPPBBO BB
PULPROG              zg30
TD                  65536
SOLVENT              MeOD
NS                     16
DS                      2
SWH              10000.000 Hz
FIDRES               0.152588 Hz
AQ                3.2767999 sec
RG                    32.16
DW                   50.000 usec
DE                   10.00 usec
TE                  298.2 K
D1                1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1            500.1530886 MHz
NUC1                   1H
P1                   15.00 usec
PLW1            12.30000019 W

F2 - Processing parameters
SI                    65536
SF              500.1500104 MHz
WDW                    EM
SSB         0
LB                    0.30 Hz
GB          0
PC                    1.00
```



BRUKER

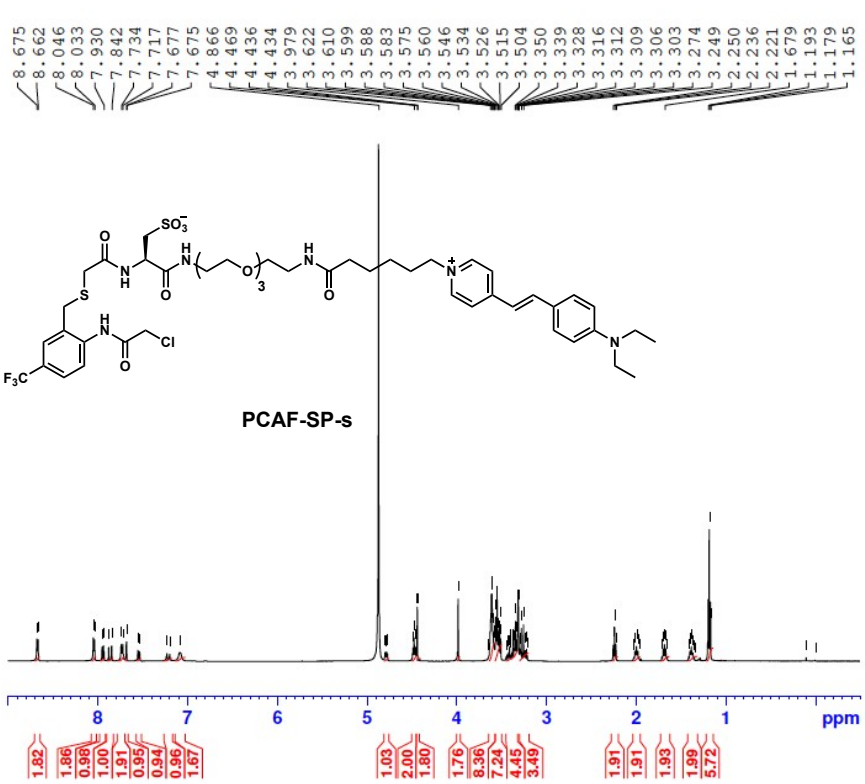
```
Current Data Parameters
NAME      20211104_No2326_comp18_13C
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_              20211104
Time                 12.39
INSTRUM              spect
PROBHD    5 mm CPPBBO BB
PULPROG            zgpg30
TD                  65536
SOLVENT              MeOD
NS                   1024
DS                      4
SWH              29761.904 Hz
FIDRES               0.454131 Hz
AQ                1.1010048 sec
RG                   197.07
DW                   16.800 usec
DE                   18.00 usec
TE                  298.1 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1            125.7753932 MHz
NUC1                   13C
P1                   10.00 usec
PLW1            77.00000000 W

======== CHANNEL f2 ========
SFO2            500.1520006 MHz
NUC2                   1H
CPDPRG[2            waltz16
PCPD2                80.00 usec
PLW2            12.30000019 W
PLW12            0.43241999 W
PLW13            0.27675000 W

F2 - Processing parameters
SI                    32768
SF              125.7628175 MHz
WDW                    EM
SSB         0
LB                    1.00 Hz
GB          0
PC                    1.40
```

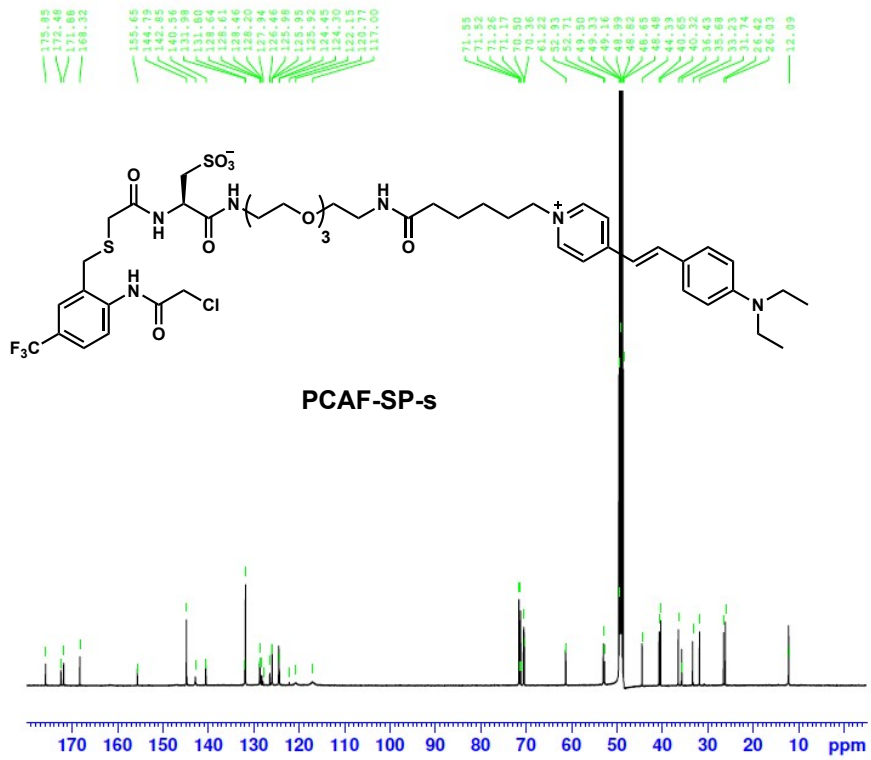



```
Current Data Parameters
NAME      20211217_PCAF-SP2
EXPNO                   10
PROCNO                   1

F2 - Acquisition Parameters
Date_            20211217
Time                11.21
INSTRUM             spect
PROBHD   5 mm CPPBBO BB
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES            0.152588 Hz
AQ                3.2767999 sec
RG                   32.16
DW                  50.000 usec
DE                  10.00 usec
TE                  298.1 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1           500.1530886 MHz
NUC1                   1H
P1                  15.00 usec
PLW1           12.30000019 W

F2 - Processing parameters
SI                 65536
SF            500.1500098 MHz
WDW                   EM
SSB          0
LB                  0.30 Hz
GB           0
PC                  1.00
```



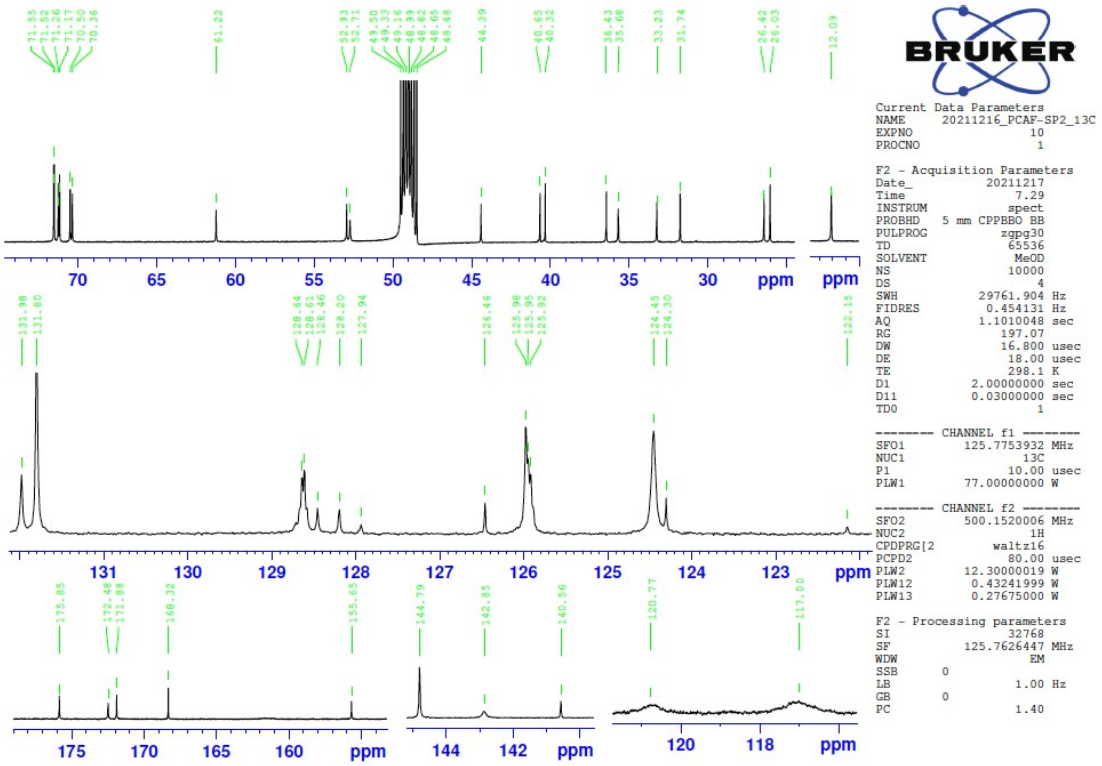

```
Current Data Parameters
NAME      20211216_PCAF-SP2_13C
EXPNO                   10
PROCNO                   1

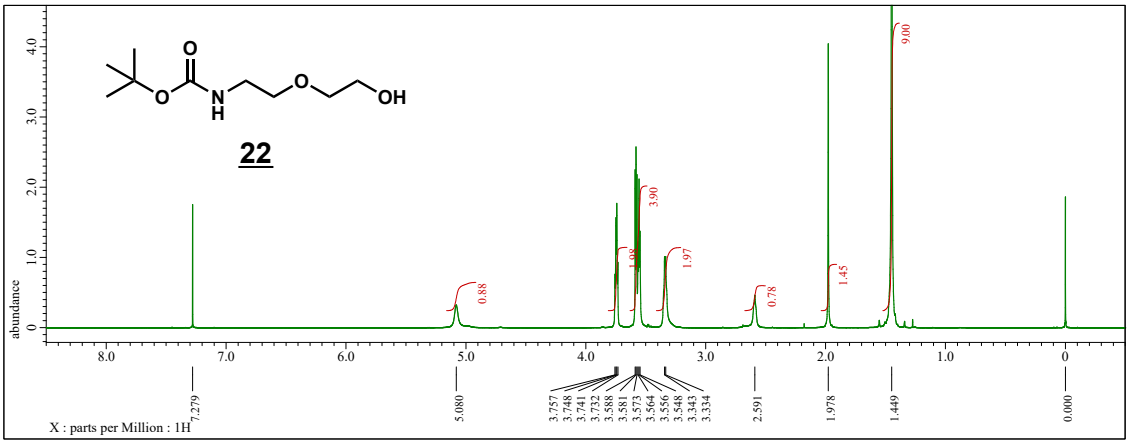
F2 - Acquisition Parameters
Date_            20211217
Time                7.29
INSTRUM             spect
PROBHD   5 mm CPPBBO BB
PULPROG          zgpg30
TD                 65536
SOLVENT             MeOD
NS                 10000
DS                     4
SWH           29761.904 Hz
FIDRES            0.454131 Hz
AQ                1.1010048 sec
RG                  197.07
DW                  16.800 usec
DE                  18.00 usec
TE                  298.1 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                     1

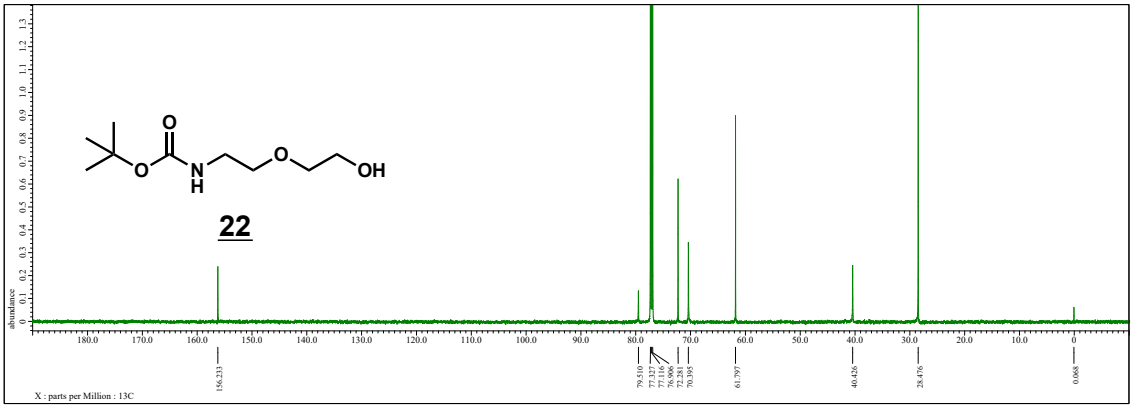
======== CHANNEL f1 ========
SFO1           125.7753932 MHz
NUC1                   13C
P1                  10.00 usec
PLW1           77.00000000 W

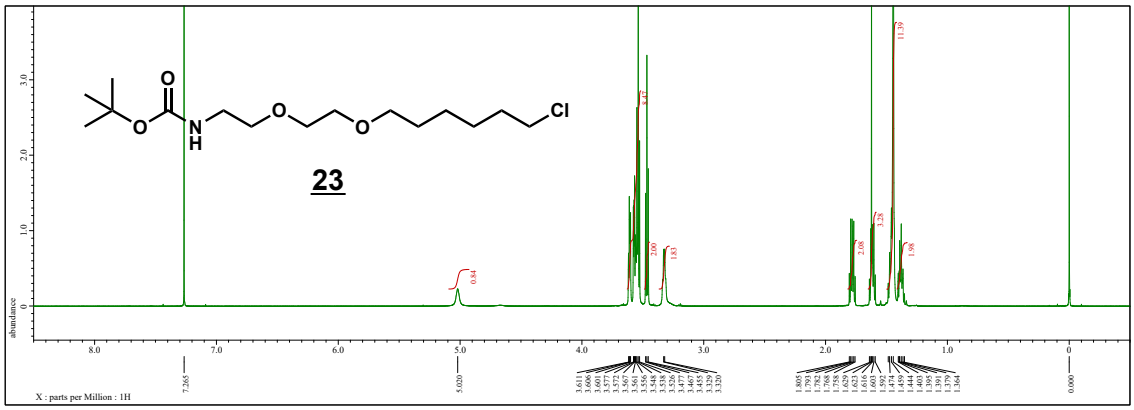
======== CHANNEL f2 ========
SFO2           500.1520006 MHz
NUC2                   1H
CPDPRG[2           waltz16
PCPD2               80.00 usec
PLW2           12.30000019 W
PLW12           0.43241999 W
PLW13           0.27675000 W

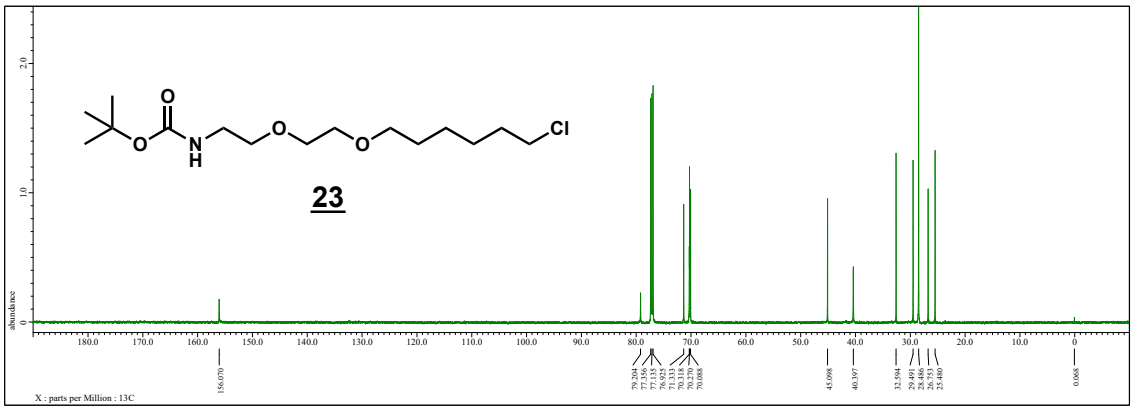
F2 - Processing parameters
SI                 32768
SF            125.7626447 MHz
WDW                   EM
SSB          0
LB                  1.00 Hz
GB           0
PC                  1.40
```

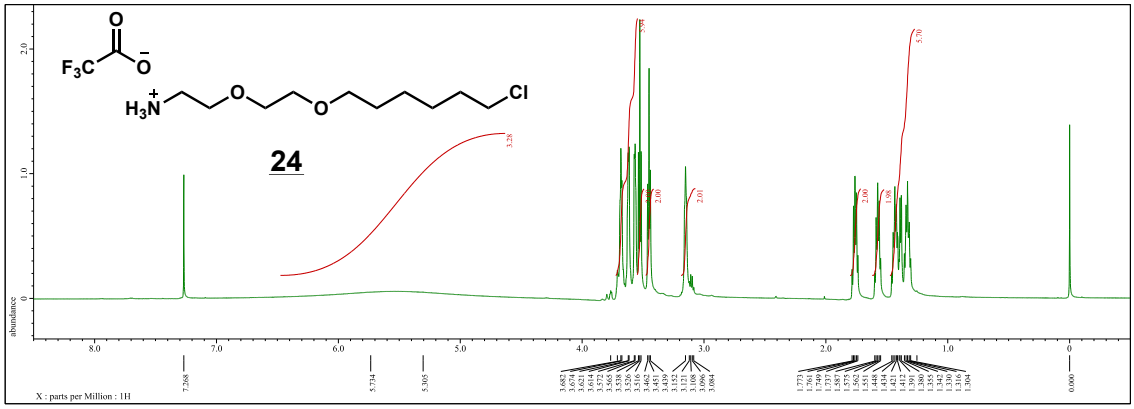

71.55
71.52
71.26
71.17
70.90
70.36
61.22
52.93
52.71
49.50
49.33
49.16
48.99
48.82
48.65
48.48
44.39
40.65
40.32
36.43
35.68
33.23
31.74
26.42
26.03
12.09
70 65 60 55 50 45 40 35 30 ppm
131.98
131.80
128.64
128.61
128.46
128.20
127.94
126.46
125.98
125.85
125.92
124.45
124.30
122.15
131 130 129 128 127 126 125 124 123 ppm
175.85
172.48
171.88
168.32
155.65
144.79
142.85
140.56
120.77
117.00
175 170 165 160 ppm
144 142 ppm
120 118 ppm
BRUKER
Current Data Parameters
NAME 20211216_PCAF-SP2_13C
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20211217
Time 7.29
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 10000
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.07
DW 16.800 usec
DE 18.00 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 125.7753932 MHz
NUC1 13C
P1 10.00 usec
PLW1 77.00000000 W
CHANNEL f2
SFO2 500.1520006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.30000019 W
PLW12 0.43241999 W
PLW13 0.27675000 W
F2 - Processing parameters
SI 32768
SF 125.7626447 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

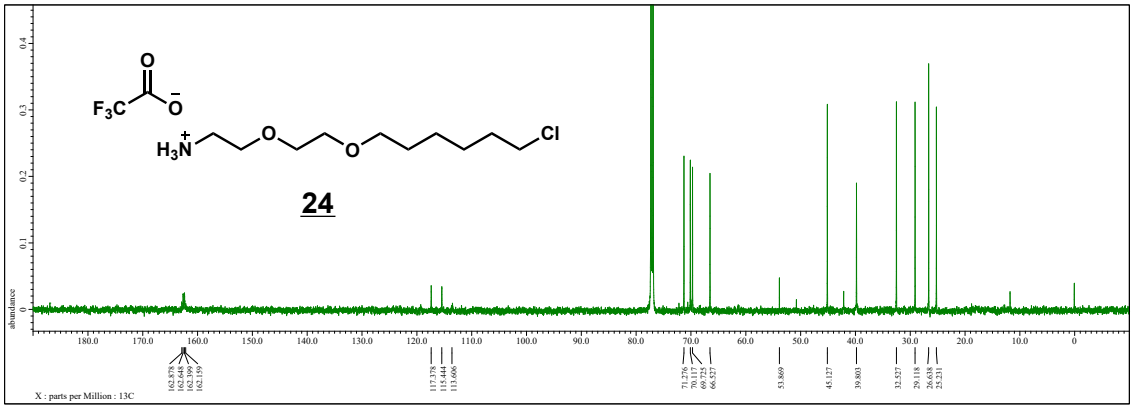

22
abundance
X : parts per Million : 1H
7.279
5.080
3.757
3.748
3.741
3.732
3.588
3.581
3.573
3.564
3.556
3.548
3.343
3.334
2.591
1.978
1.449
0.000
0.88
3.90
1.97
0.78
1.45
9.00

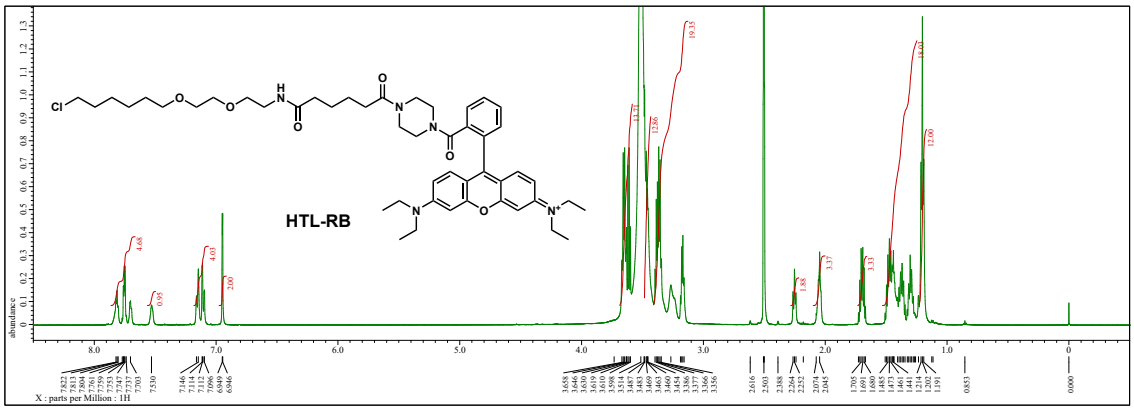

22
abundance
X : parts per Million : 13C
156.233
79.510
77.327
77.146
76.968
72.281
70.395
61.797
40.426
28.476
0.068

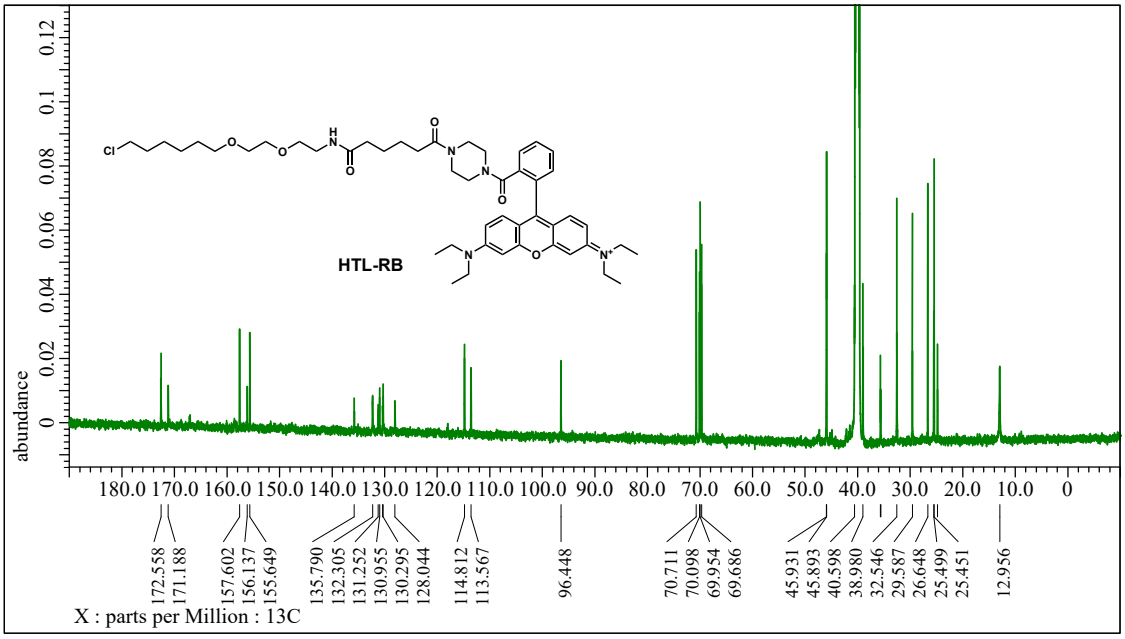

23
abundance
X : parts per Million : 1H

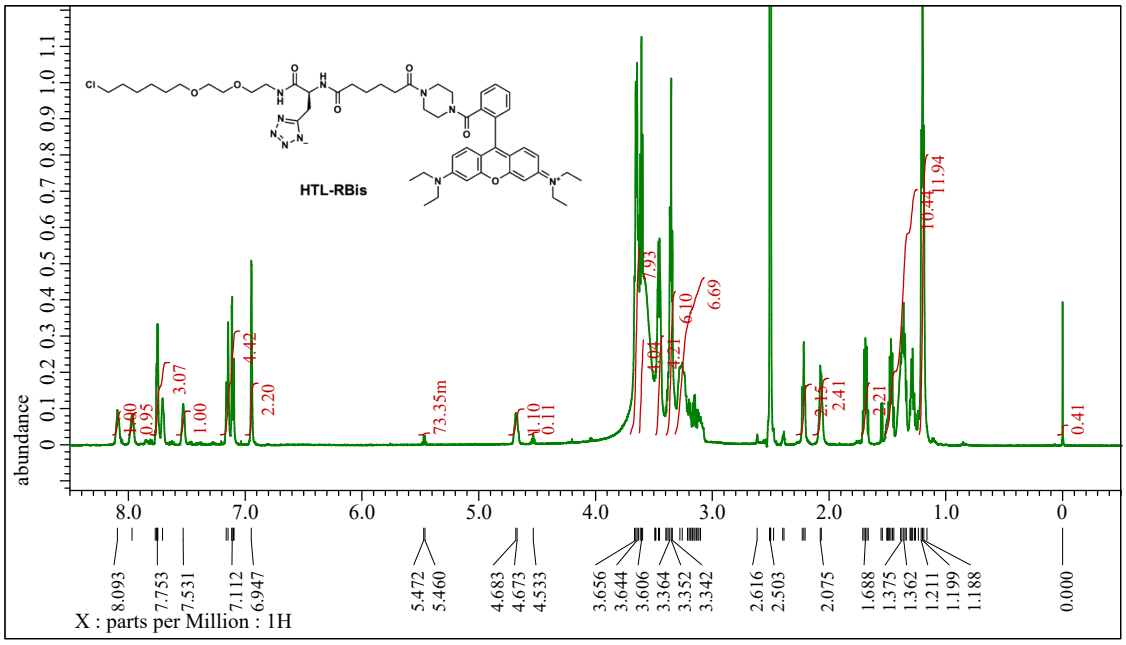

23
abundance
X : parts per Million : 13C

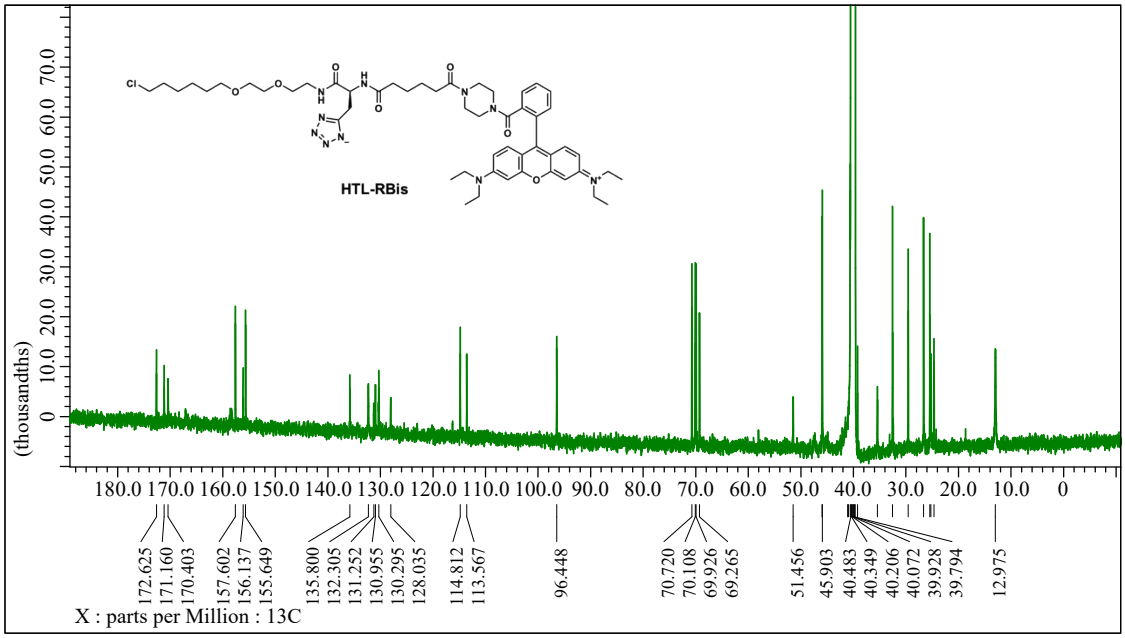

24
X : parts per Million : 1H

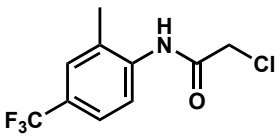

24
X : parts per Million : 13C


HTL-RB
abundance
X : parts per Million : 1H

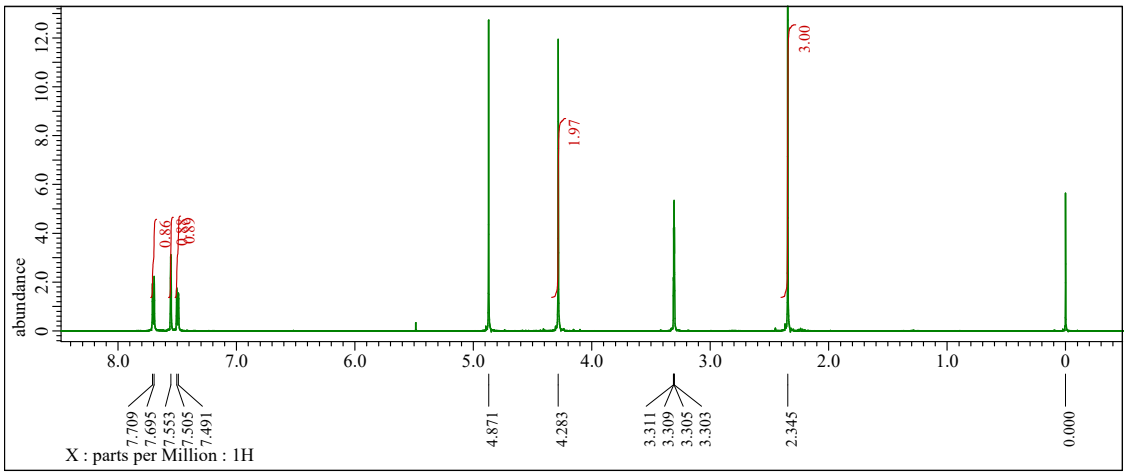

HTL-RB
abundance
172.558
171.188
157.602
156.137
155.649
135.790
132.305
131.252
130.955
130.295
128.044
114.812
113.567
96.448
70.711
70.098
69.954
69.686
45.931
45.893
40.598
38.980
32.546
29.587
26.648
25.499
25.451
12.956
X : parts per Million : 13C

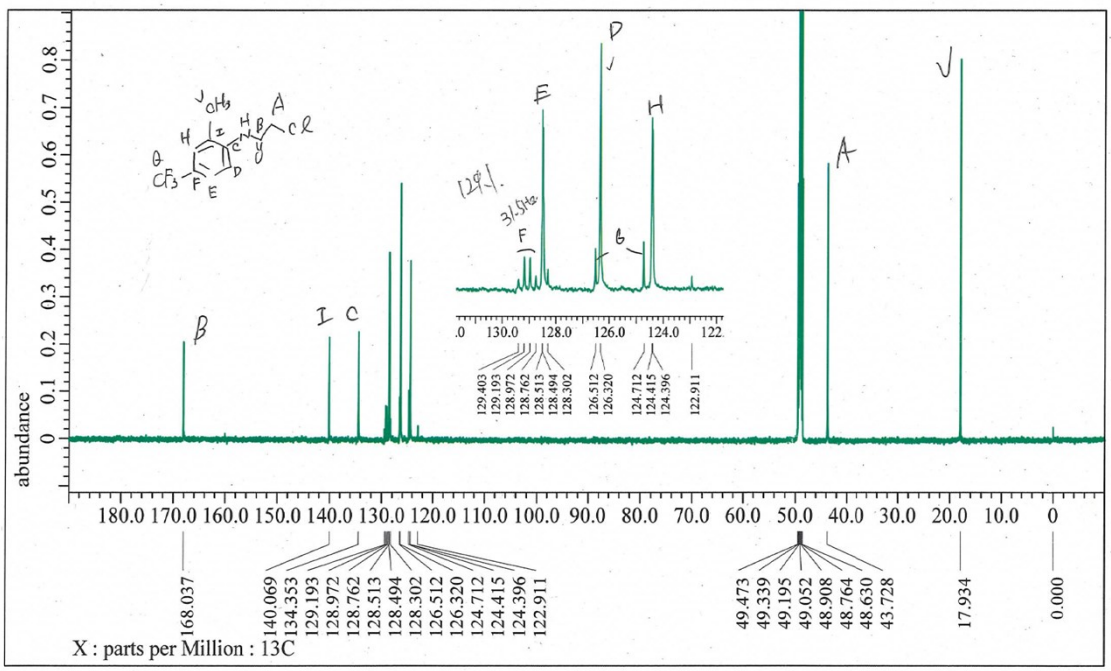

HTL-RBis
abundance
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1
8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0
1.40
0.95
3.07
1.00
4.42
2.20
73.35m
4.10
0.11
7.93
4.04
4.21
6.10
6.69
2.15
2.41
3.21
10.44
11.94
0.41
8.093 7.753 7.531 7.112 6.947 5.472 5.460 4.683 4.673 4.533 3.656 3.644 3.606 3.364 3.352 3.342 2.616 2.503 2.075 1.688 1.375 1.362 1.211 1.199 1.188 0.000
X : parts per Million : 1H


HTL-RBis
(thousandths)
0 10.0 20.0 30.0 40.0 50.0 60.0 70.0
180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
172.625 171.160 170.403 157.602 156.137 155.649 135.800 132.305 131.252 130.955 130.295 128.035 114.812 113.567 96.448 70.720 70.108 69.926 69.265 51.456 45.903 40.483 40.349 40.206 40.072 39.928 39.794 12.975
X : parts per Million : 13C

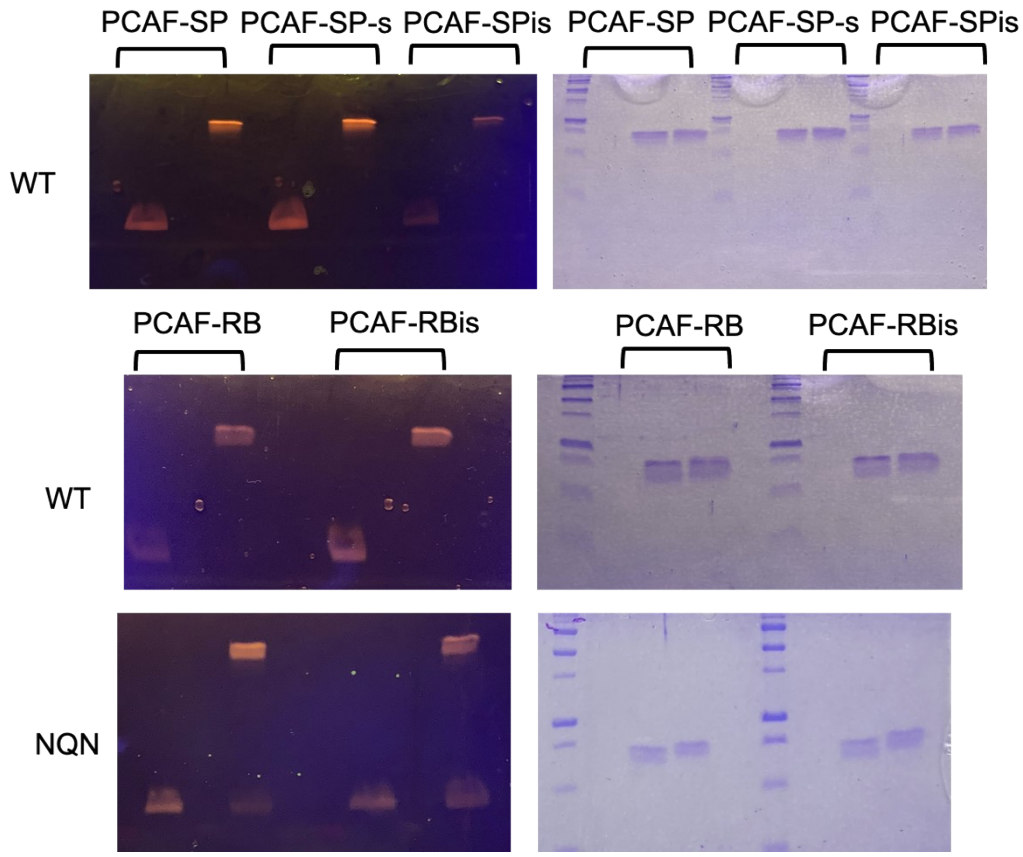

H
N
Cl
O
F3C

PCAF-Me

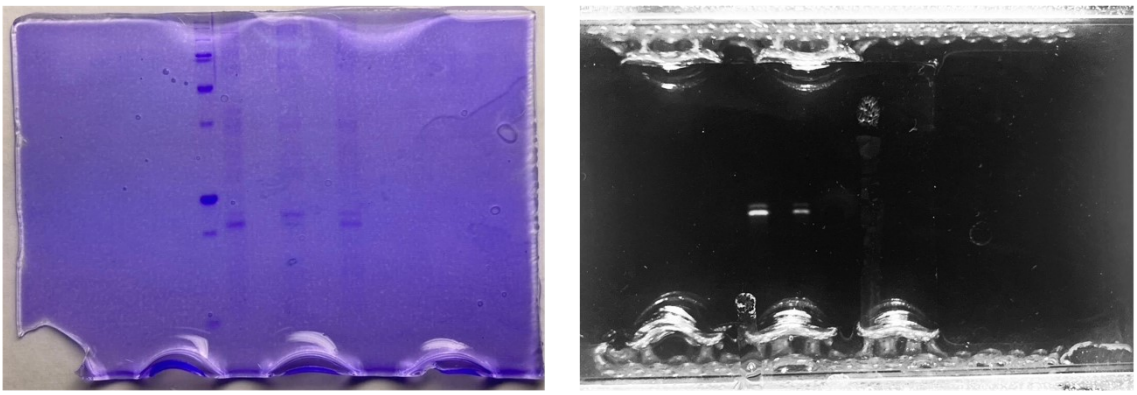

abundance
3.00
1.97
0.86
X : parts per Million : 1H
7.709
7.695
7.553
7.505
7.491
4.871
4.283
3.311
3.309
3.305
3.303
2.345
0.000


abundance
X : parts per Million : 13C
168.037
140.069
134.353
129.193
128.972
128.762
128.513
128.494
128.302
126.512
126.320
124.712
124.415
124.396
122.911
49.473
49.339
49.195
49.052
48.908
48.764
48.630
43.728
17.934
0.000

6. The Raw Images of Electrophoresis Data



The raw images of electrophoresis data in Fig. 2b



The raw images of electrophoresis data in Fig. S4

7. References

S1 M. Nishiura, Y. Hori, M. Umeno and K. Kikuchi, *Chem. Sci.*, 2023, **14**, 5925-5935.

S2 Y. Hori, H. Ueno, S. Mizukami and K. Kikuchi, *J. Am. Chem. Soc.*, 2009, **131**, 16610–16611.

S3 Y. Hori, K. Nakaki, M. Sato, S. Mizukami and K. Kikuchi, *Angew. Chem. Int. Ed.*, 2012, **51**, 5611-5614.

S4 Y. Hori, T. Norinobu, M. Sato, K. Arita, M. Shirakawa, and K. Kikuchi, *J. Am. Chem. Soc.*, 2013, **135**, 12360-12365.

S5 S. I. Reja, Y. Hori, T. Kamikawa, K. Yamasaki, M. Nishiura, S. D. Bull and K. Kikuchi, *Chem. Sci.*, 2022, **13**, 1419-1427.

S6 A. M. Brouwer, *Pure Appl. Chem.,* **2011**, *83*, 2213–2228.

S7 X. F. Zhang, Y. Zhang and L.Liu, *J. Lumin.* **2014**, *145*, 448-453.