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Supplementary information

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A palette of bridged bicycle-strengthened fluorophores

In the format provided by the authors and unedited

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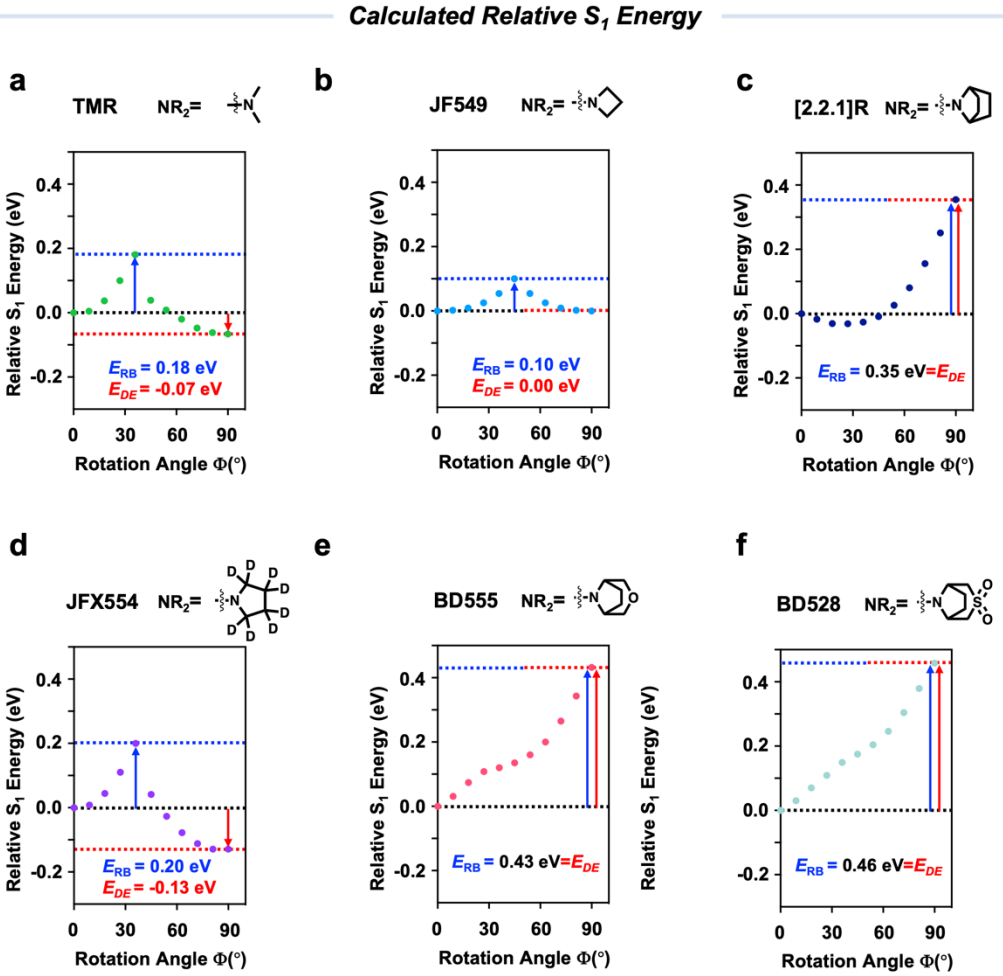


Fig.S1 Calculated relative S_1 energy for O-rhodamine with various auxochromes in water. (a) **TMR**; (b) **JF549**; (c) **[2.2.1]R**; (d) **JFX554**; (e) **BD555**; (f) **BD528**.

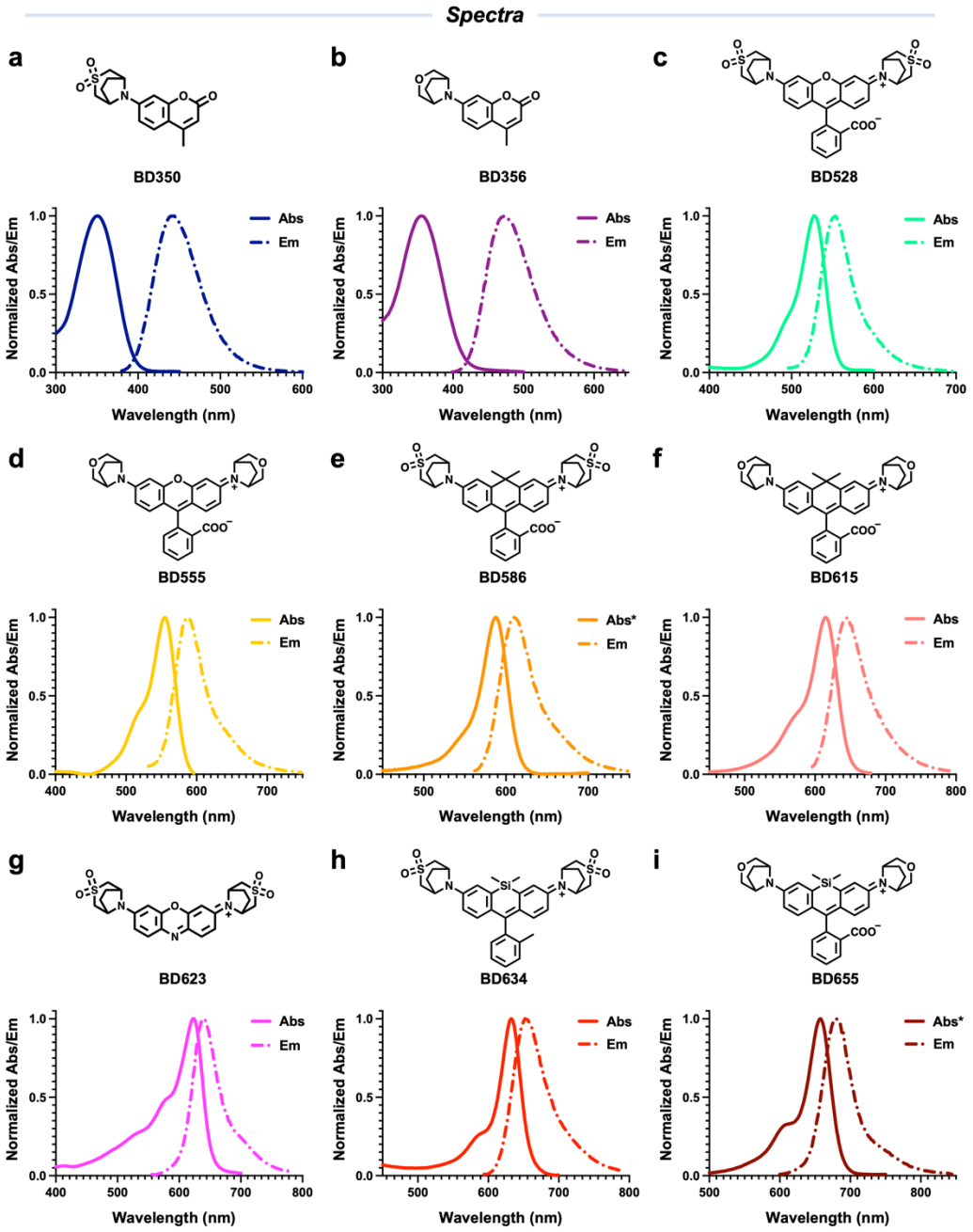


Fig.S2 Spectra for BD dyes.

Normalized absorption (Abs) and fluorescence emission (Em) spectra for (a) **BD350**; (b) **BD356**; (c) **BD528**; (d) **BD555**; (e) **BD586**; (f) **BD615**; (g) **BD623**; (h) **BD634**; (i) **BD655** in HEPES buffer, (10 mM, pH=7.3) (*n*=2).

*Absorption of **BD586** in (e) and **BD655** in (i) were measured in HEPES buffer (10 mM, pH=7.3) with 0.1% SDS to enforce the open-zwitterionic form.

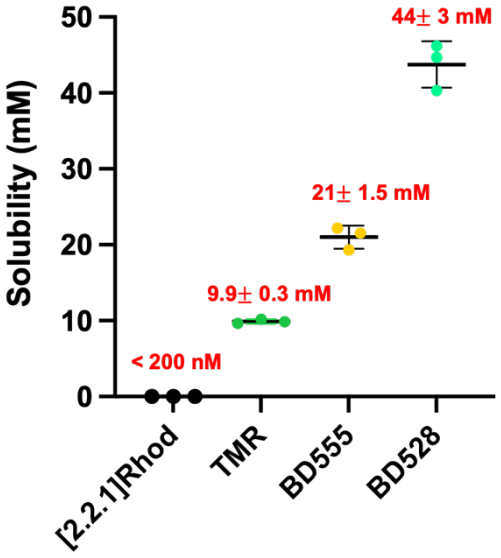


Fig.S3 Water solubility of [2.2.1]Rhod, TMR, BD555 and BD528.
Each dot represents an independent experiment; $n = 3$; data are presented as mean ± S.D.

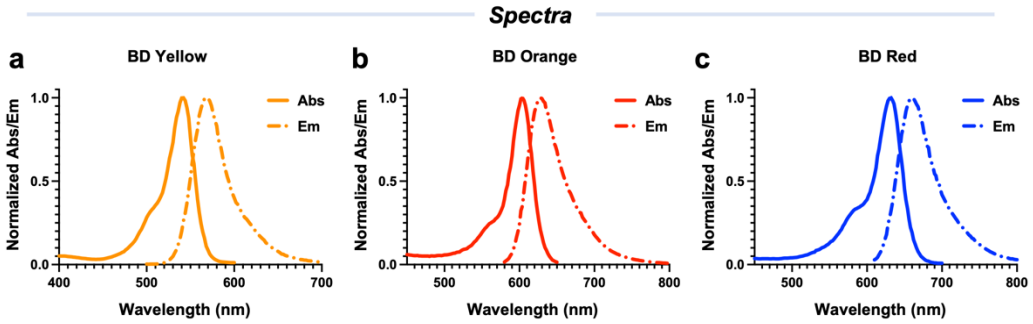
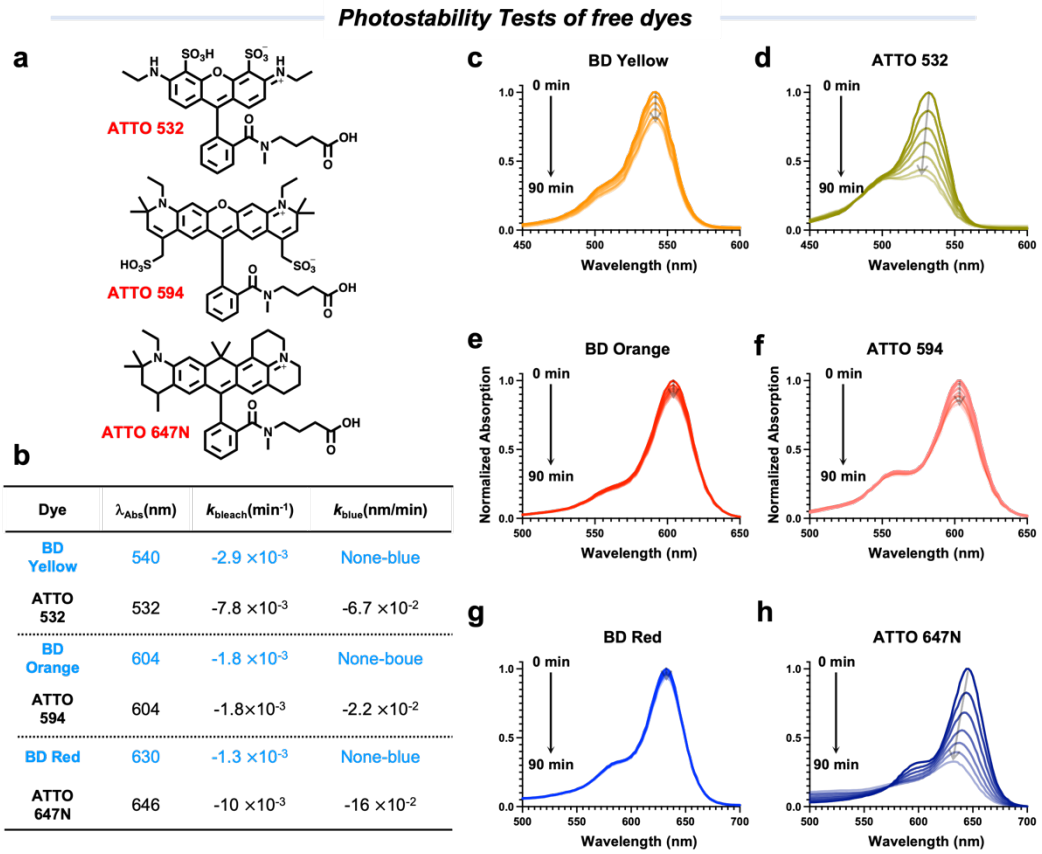


Fig.S4 Spectra for BD derivatives for antibody bioconjugation.
Normalized absorption (Abs) and fluorescence emission (Em) spectra for (a) **BD Yellow**; (b) **BD Orange**; (c) **BD Red** in HEPES buffer (10 mM, pH=7.3) (n=2 independent experiments).



Dye	λ_{Abs}(nm)	k_{bleach}(min^{-1})	k_{blue}(nm/min)
BD Yellow	540	-2.9 ×10^{-3}	None-blue
ATTO 532	532	-7.8 ×10^{-3}	-6.7 ×10^{-2}
BD Orange	604	-1.8 ×10^{-3}	None-boue
ATTO 594	604	-1.8×10^{-3}	-2.2 ×10^{-2}
BD Red	630	-1.3 ×10^{-3}	None-blue
ATTO 647N	646	-10 ×10^{-3}	-16 ×10^{-2}

Fig.S5 Photostability and photoblue properties of free BD derivatives for antibody bioconjugation and various ATTO dyes.
(a) Chemical structures of various **ATTO** dyes;
(b) Photobleaching and photobluing rates of selected fluorophores in HEPES buffer (10 mM, pH 7.3) (*n*=3 independent experiments);
(c) to (d) The absorption of dyes over time demonstrates the photobleaching of photooxidation-resistant, nonbluing fluorophore **BD Yellow** (c) in comparison with photooxidation-prone fluorophore **ATTO 532** (d) under irradiation with an LED lamp (0.5 W/cm^2, 520-530 nm).
(e) to (f) The absorption of dyes over time demonstrates the photobleaching of photooxidation-resistant, nonbluing fluorophore **BD Orange** (e) in comparison with photooxidation-prone fluorophore **ATTO 594** (d) under irradiation with an LED lamp (0.5 W/cm^2, 590-600 nm).
(g) to (h) The absorption of dyes over time demonstrates the photobleaching of photooxidation-resistant, nonbluing fluorophore **BD Red** (g) in comparison with photooxidation-prone fluorophore **ATTO 647N** (h) under irradiation with an LED lamp (0.5 W/cm^2, 620-630 nm).

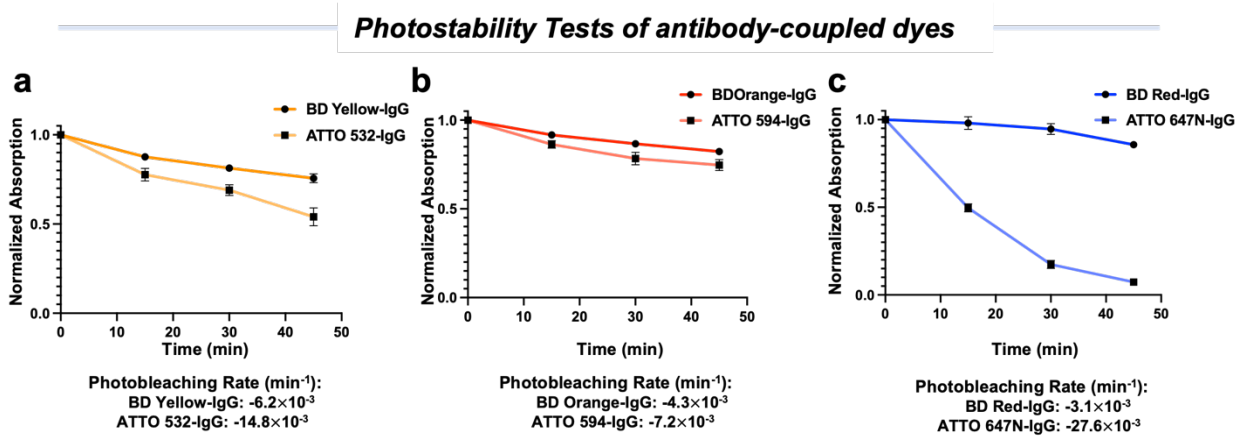


Fig.S6 Photostability of antibody-coupled BD derivatives and selected ATTO dyes.
(a) to (c) Photobleaching curves of antibody-coupled dyes in HEPES buffer (10 mM, pH=7.3), (*n*=3 independent experiments, error bar show mean± S.D.). (a) Absorption at λ_{max} of **BD Yellow** (DOL=3.27) and **ATTO 532** (DOL=3.47)were plotted as a function of irradiation time with an LED lamp (0.5 W/cm^2) at 520-530 nm; (d) Absorption at λ_{max} of **BD Orange** (DOL=2.63)and **ATTO 594** (DOL=2.50) were plotted as a function of irradiation time with an LED lamp (0.5 W/cm^2) at 590-600 nm; (e) Absorption at λ_{max} of **BD Red** (DOL=2.42) and **ATTO 647N** (DOL=2.02) were plotted as a function of irradiation time with an LED lamp (0.5 W/cm^2) at 620-630 nm.

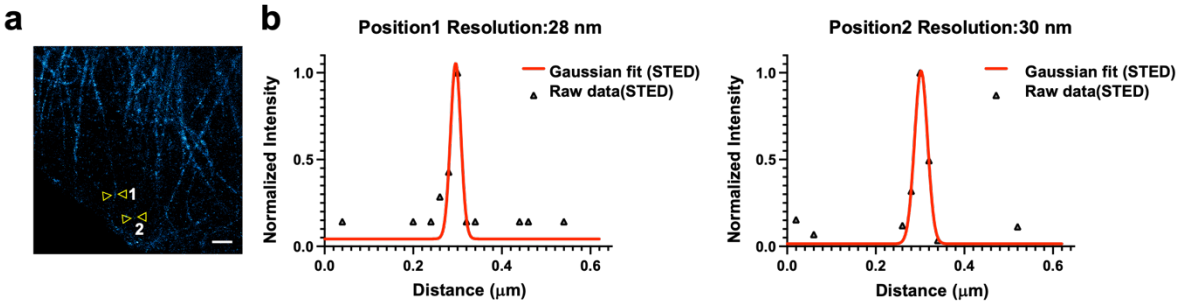


Fig.S7 Ultra-high resolution immunofluorescence STED imaging by BD Red.
(a) STED images of α-tubulin structures in fixed-HeLa cells labeled by indirect immunofluorescence with a secondary antibody bearing **BD Red** (DOL=2.42), 640 nm Ex./650-700 nm Em., STED at 775 nm (~200 mW), scale bar=1 μm; this experiment was independently repeated three times;
(b) Line-scan profile of fluorescence intensity at the yellow arrow in (a) under ultra-high resolution STED conditions.

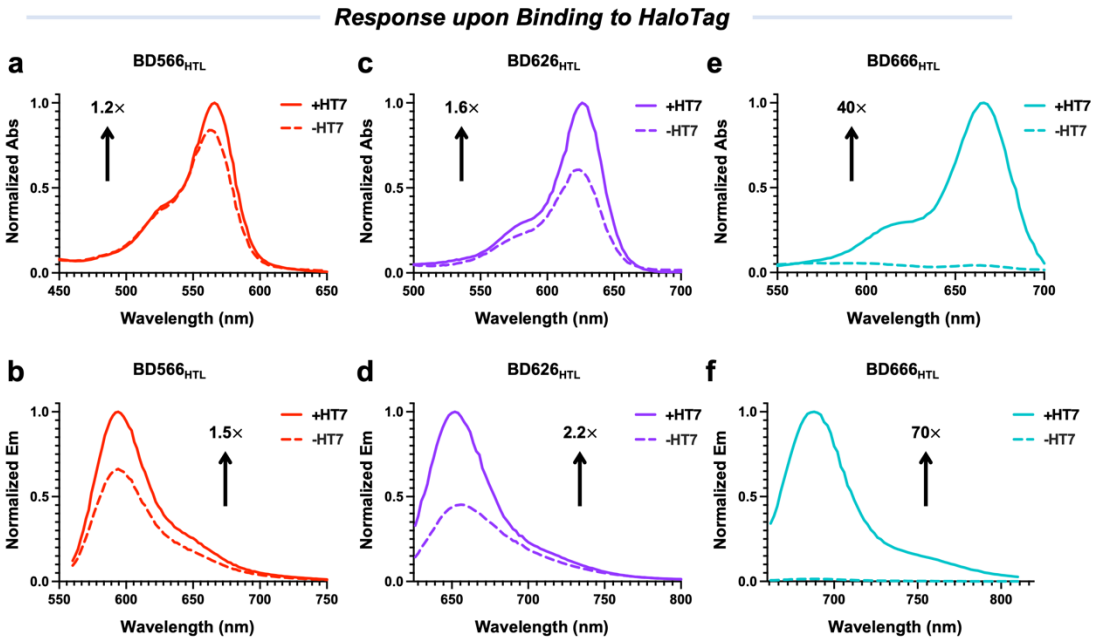


Fig.S8 Response of BD$_{HTL}$s to HaloTag.

Absorption (a, c, e) and fluorescence emission (b, d, f) spectra of 2 μM of **BD566$_{HTL}$** (a-b), **BD626$_{HTL}$** (c-d), **BD666$_{HTL}$** (e-f) measured in the absence (dashed line) and presence of HaloTag (5 μM, solid line) after 2 h incubation in HEPES buffer (10 mM, pH 7.3) (*n*=3 independent experiments). The numbers indicate the ratio of absorption maximum or emission maximum of each **BD$_{HTL}$**s in the presence and absence of HaloTag.

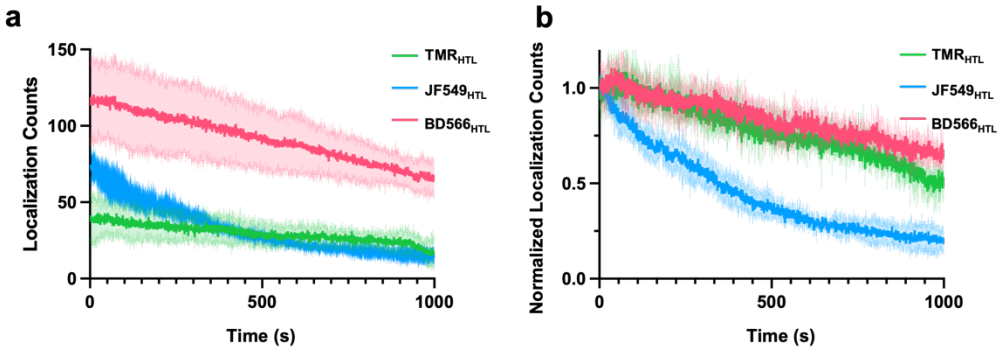


Fig. S9 Single-molecule localization number per nuclei over time. (a) Original data; (b) Normalized data, with the initial number as 1. n=4 cells in 2 independent experiments; error bar show mean ± S.D.

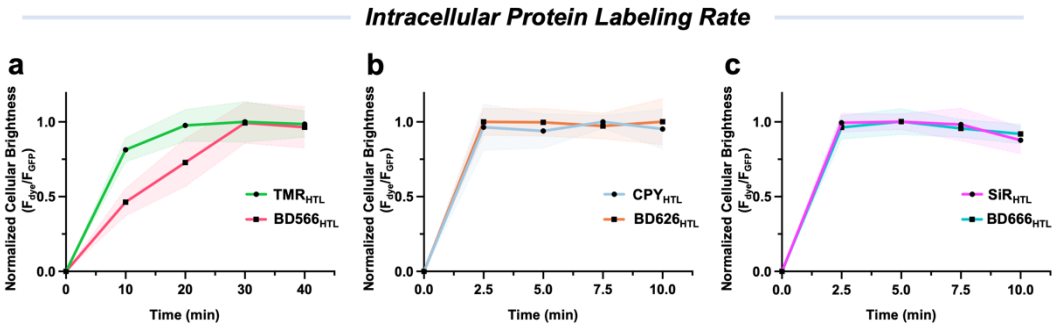


Fig.S10 Intracellular protein labeling rate of BD_{HTL}s and control dyes.
Normalized ratio of **BD_{HTL}s** and control ligands (200 nM each) fluorescence to GFP fluorescence at different time point in HeLa cells stably expressing H2B-HaloTag7-GFP; *n*=100 cells in each group were examined in 2 independent experiments; error bar show mean± S.D.
(a) $BD566_{HTL}$ v. s. **TMR_{HTL}**; (b) **$BD626_{HTL}$** v. s. **CPY_{HTL}**; (c) **$BD666_{HTL}$** v. s. **SiR_{HTL}**.

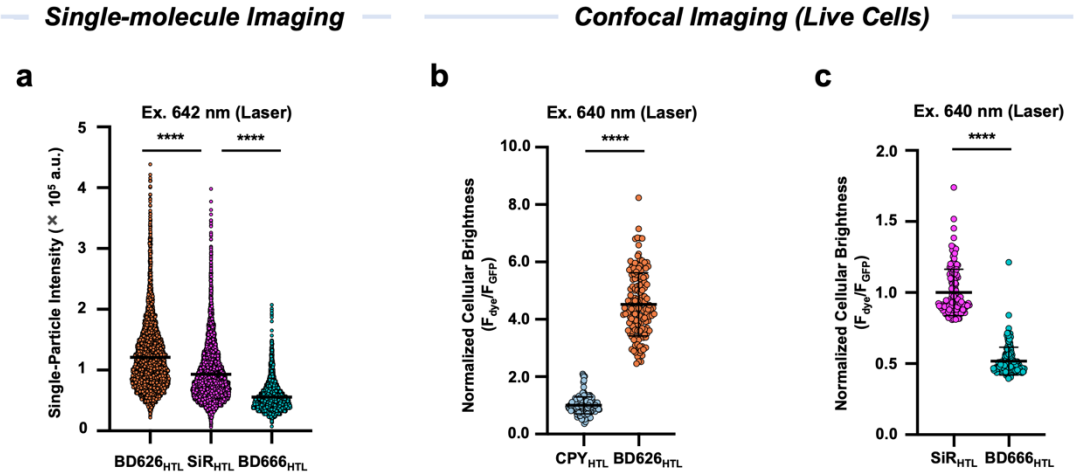


Fig.S11 Brightness of BD_{HTL}s and control ligands on mammalian cells under 633/640-nm lasers.
(a) Comparison of brightness on single-molecule level in fixed U-2 OS cells stably expressing H2B-HaloTag7 labeled with **$BD626_{HTL}$**, **SiR_{HTL}** and **$BD666_{HTL}$** (2.5 pM, 30 min, 37°C, one wash) under 642-nm laser;
For **$BD626_{HTL}$**, *n*= 5158; **SiR_{HTL}**,*n*= 5591; **$BD666_{HTL}$**, *n*= 4476; *p*=1.319E-179 (**$BD626_{HTL}$** v.s. **SiR_{HTL}**); *p*=5.656E-183 (**SiR_{HTL}** v.s. **$BD666_{HTL}$**); error bar show mean± S.E.M.
(b-c) Comparison of apparent cellular brightness in live HeLa cells stably expressing H2B-HaloTag7-GFP labeled with different HaloTag ligands (200 nM, 1 h, 37°C, one wash) under 640-nm laser. The fluorescence intensity from single nuclei was normalized by the cytosolic GFP fluorescence intensity of the same cell (expression control) to assess the cellular brightness (error bar show mean ± S.D.);
For **CPY_{HTL}**, *n*=154 cells, **$BD626_{HTL}$**, *n*=149 cells; *p*=2.121E-117;
For **SiR_{HTL}**, *n*=150 cells, for **$BD666_{HTL}$**, *n*=158 cells; *p*=7.913E-99;
Significance was evaluated by a two-sided unpaired Student's t-test for mean. *p**<0.0001.

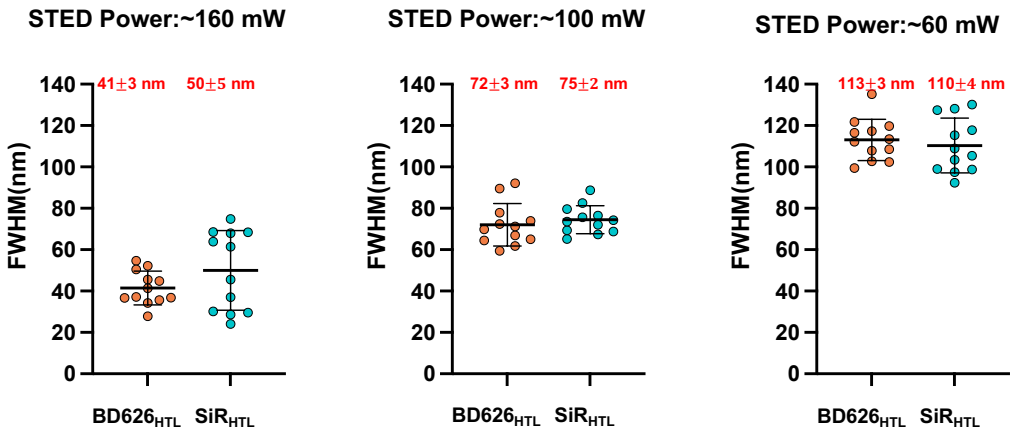


Fig.S12 Comparison of the FWHM achieved under different STED microscopy conditions. Live HeLa cells expressing HT7-Cep41 and stain with **BD626$_{HTL}$** or **SiR$_{HTL}$** (500 nM, 30 min, 37°C, one wash). (n=12 filements from 3 samples).

Table S1 Statistical tests and exact *p* values for Fig 4.

Figure Index	Comparision group	Statistical test	*P* value	Symbol
Figure 4e	TMR$_{HTL}$ vs JF549$_{HTL}$	Two-sided unpaired students t-test	6.666E-126	****
Figure 4e	JF549$_{HTL}$ vs BD566$_{HTL}$	Two-sided unpaired students t-test	4.259E-119	****
Figure 4f	TMR$_{HTL}$ vs JF549$_{HTL}$	Two-sided unpaired students t-test	2.762E-35	****
Figure 4f	JF549$_{HTL}$ vs BD566$_{HTL}$	Two-sided unpaired students t-test	1.017E-13	****
Figure 4h	TMR$_{HTL}$ vs JF549$_{HTL}$	Two-sided unpaired students t-test	2.598E-131	****
Figure 4h	JF549$_{HTL}$ vs BD566$_{HTL}$	Two-sided unpaired students t-test	1.744E-29	****
Figure 4j	CPY$_{HTL}$ vs BD626$_{HTL}$	Two-sided unpaired students t-test	1.062E-101	****
Figure 4n	SiR$_{HTL}$ vs BD666$_{HTL}$	Two-sided unpaired students t-test	4.713E-63	****
Figure 4r	BD566$_{HTL}$ vs JF552$_{HTL}$	Two-sided unpaired students t-test	0.4059	ns

Table S2 Data collection and refinement statistics for the crystal structure of BD626$_{HTL}$-HaloTag7. Values in parentheses are for the highest resolution shell.

PDB code	
Data-collection statistics	
Diffraction Source	XtaLAB Synergy Custom FRX
Wavelength (Å)	1.54178
Temperature (K)	100
Resolution range (Å)	31.35 - 1.698 (1.759 - 1.698)
Space group	$P4_32_12$
Unit cell parameters	
a, b, c (Å)	62.696, 62.696, 164.386
α, β, γ (°)	90, 90, 90
Total reflections	1004475
Unique reflections	37009 (3540)
Multiplicity	27.1(4.9)
Completeness (%)	99.55 (97.84)
Mean I/σ (*I*)	39.47(1.70)
Wilson *B* factor (Å^2)	21.37
R_{meas}	0.126(0.604)
$R_{p.i.m.}$	0.020(0.256)
$CC_{1/2}$	0.998(0.859)
Structure-refinement statistics	
Reflections used in refinement	36979 (3540)
Reflections used for R_{free}	1841 (170)
R_{work}	0.1912 (0.2334)
R_{free}	0.2404 (0.2723)
No. of protein chains in asymmetric unit	1
No. of non-H atoms	2857
Protein residues	299
R.m.s.d., bond lengths (Å)	0.047
R.m.s.d., bond angles (°)	0.9
Ramachandran statistics (%)	
Favored	95.53
Allowed	4.47
Outliers	0
Rotamer outliers (%)	0
Clashscore	1.28
Average *B* factor (Å^2)	
Overall	24.46
Macromolecules	22.27
Ligands	35.42
Solvent	34.26

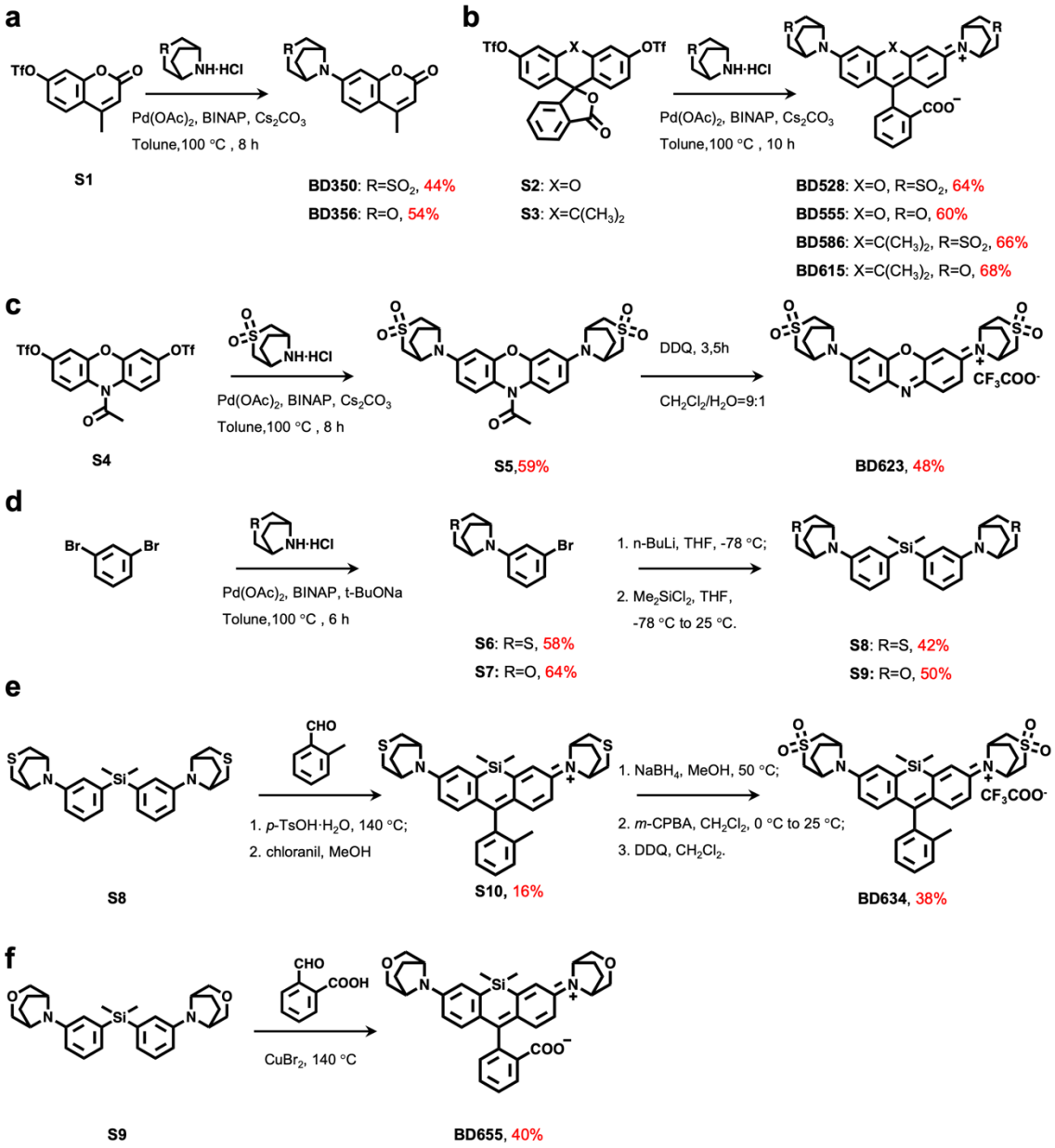
Synthesis and Characterization of New Compounds

General Information

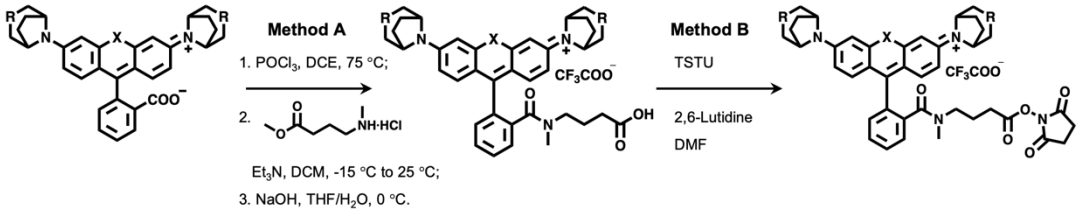
Unless otherwise mentioned, all reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions. All the chemicals were purchased at the highest commercial quality and used without further purification unless otherwise stated. Reactions were conducted in round-bottomed flasks or septum-capped crimp-top vials containing Teflon-coated magnetic stir bars. The heating of reactions were accomplished with a silicon oil bath on a stirring hotplate equipped with an electronic contact thermometer to maintain the indicated temperatures. Anhydrous N, N-dimethylformamide (DMF) and tetrahydrofuran (THF) was purchased from Innochem (China). 3-thia-8-azabicyclo [3.2.1] octane 3,3-dioxide hydrochloride, 3-thia-8-azabicyclo [3.2.1] octane 3,3-dioxide hydrochloride and 3-thia-8-azabicyclo [3.2.1] octane hydrochloride were perchased from PharmaBlock (China).

Reactions were monitored by Thin Layer Chromatography on plates (GF254) supplied by Yantai Chemicals (China) using UV light as a visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate, and heat as developing agents or by LC/MS (4.6 mm × 150 mm 5 μm C18 column; 2 μL injection; 5-100% CH_3CN/H_2O, linear-gradient, with constant 0.1% v/v TFA additive; 6 min run; 0.6 mL/min flow; ESI; positive ion mode; UV detection at 254 nm with ACQUITY PDA). If not specially mentioned, flash column chromatography uses silica gel (200-300 mesh) supplied by Tsingtao Haiyang Chemicals (China). Preparative HPLC separations were performed using Teledyne Isco EZ Prep UV-Vis and a RediSep Prep C18 column (100 Å, 5 μm, 20 × 150 mm).

NMR spectra were recorded on Brüker Advance 400 (^{1}H 400 MHz, ^{13}C 101 MHz) and are calibrated using residual undeuterated solvent ($CDCl_3$ at 7.26 ppm ^{1}H NMR, 77.16 ppm ^{13}C NMR; CD_3OD at 3.31 ppm ^{1}H NMR, 49.00 ppm ^{13}C NMR; CD_3CN at 1.94 ppm ^{1}H NMR, 1.32 and 118.26 ppm ^{13}C NMR; DMSO-d_6 at 2.50 ppm ^{1}H NMR, 39.52 ppm ^{13}C NMR). Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = triplet of doublets, m=multiplet, br=broad), coupling constant (Hz), integration. Data for ^{13}C NMR are reported by chemical shift (δ ppm). NMR data were collected by Bruker Topspin 4.2.0 and analysed by MestReNova 12.0.2. High-resolution mass spectrometric data were obtained using Acquity I class UPLC synapt G2-SI using ESI (electrospray ionization).



Scheme S1. Synthesis of bridged-bicycle strengthened fluorophores.

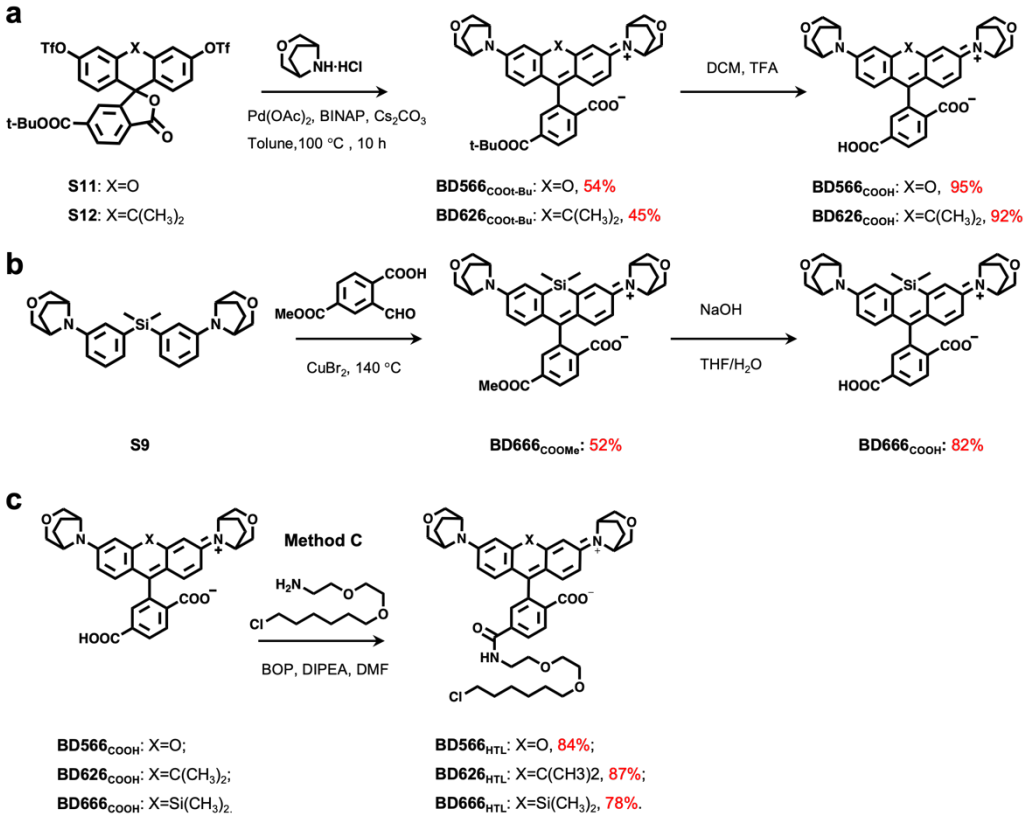


BD528: X=O, R=SO_2;
BD586: X=$C(CH_3)_2$, R=SO_2;
BD615: X=$C(CH_3)_2$, R=O.

BD Yellow: X=O, R=SO_2, 72%;
BD Orange: X=$C(CH_3)_2$, R=SO_2, 78%;
BD Red: X=$C(CH_3)_2$, R=O, 80%.

BD Yellow$_{NHS}$: X=O, R=SO_2, 80%;
BD Orange$_{NHS}$: X=$C(CH_3)_2$, R=SO_2, 76%;
BD Red$_{NHS}$: X=$C(CH_3)_2$, R=O, 78%.

Scheme S2. Synthesis of BD derivatives for bioconjugation.



S11: X=O
S12: X=$C(CH_3)_2$

BD566$_{COOt\text{-}Bu}$: X=O, 54%
BD626$_{COOt\text{-}Bu}$: X=$C(CH_3)_2$, 45%

BD566$_{COOH}$: X=O, 95%
BD626$_{COOH}$: X=$C(CH_3)_2$, 92%

S9

BD666$_{COOMe}$: 52%

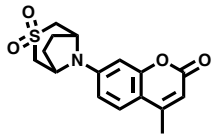
BD666$_{COOH}$: 82%

BD566$_{COOH}$: X=O;
BD626$_{COOH}$: X=$C(CH_3)_2$;
BD666$_{COOH}$: X=$Si(CH_3)_2$.

BD566$_{HTL}$: X=O, 84%;
BD626$_{HTL}$: X=C(CH3)2, 87%;
BD666$_{HTL}$: X=$Si(CH_3)_2$, 78%.

Scheme S3. Synthesis of cell-permeant BD derivatives with HaloTag ligands

Synthetic Procedures and Characterizations

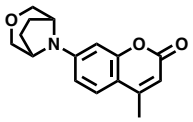


BD 350: S1 is prepared according to a published protocol[1]. A vial was charged with 4-methylumbelliferone triflate **S1** (120 mg, 390 μmol, 1.0 eq.), $Pd(OAc)_2$ (18 mg, 78 μmol, 0.2 eq.), BINAP (72 mg, 117 μmol, 0.3 eq.), Cs_2CO_3 (356 mg, 1092 μmol, 2.8 eq.) and 3-thia-8-azabicyclo [3.2.1] octane 3,3-dioxide hydrochloride (323 mg, 1640 μmol, 4.2 eq.). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (3 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 8 h. After cooling to room temperature, water (10 mL) was added and CH_2Cl_2 (10 mL × 3) was used to extract organic compounds. The combined organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the residue by silica gel chromatography (PE/EA=1/1, v/v) provided **BD 350** (55 mg, 44% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (d, J = 8.8 Hz, 1H), 6.98 (dd, J = 8.8, 2.5 Hz, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.11 – 6.07 (m, 1H), 4.93 – 4.87 (m, 2H), 3.31 – 3.28 (m, 2H), 3.27 – 3.20 (m, 2H), 2.37 (d, J = 1.2 Hz, 3H), 2.36 – 2.33 (m, 2H), 2.18 – 2.13 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 160.4, 155.3, 153.5, 146.9, 126.9, 111.6, 110.9, 109.8, 101.2, 55.0, 53.1, 26.3, 18.0.

HRMS (ESI) calcd for $C_{16}H_{18}NO_4S$ $[M+H]^+$ 320.0957, found 320.0962.

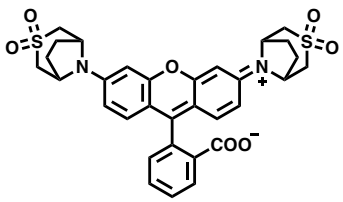


BD 356: S1 is prepared according to a published protocol[1]. A vial was charged with 4-methylumbelliferone triflate **S1** (120 mg, 390 μmol, 1.0 eq.), $Pd(OAc)_2$ (18 mg, 78 μmol, 0.2 eq.), BINAP (72 mg, 117 μmol, 0.3 eq.), Cs_2CO_3 (356 mg, 1092 μmol, 2.8 eq.) and 3-oxa-8-azabicyclo[3.2.1]octane hydrochloride (245 mg, 1640 μmol, 4.2 eq.). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (3 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 8 h. After cooling to room temperature, water (10 mL) was added and CH_2Cl_2 (10 mL × 3) was used to extract organic compounds. The combined organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the residue by silica gel chromatography (PE/EA=1/1, v/v) provided **BD 356** (57 mg, 54% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.8 Hz, 1H), 6.67 (dd, J = 8.8, 2.4 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.07 – 5.96 (m, 1H), 4.17 – 4.11 (m, 2H), 3.89 – 3.81 (m, 2H), 3.59 – 3.52 (m, 2H), 2.36 (d, J = 1.2 Hz, 3H), 2.20 – 2.03 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.0, 156.0, 152.9, 149.8, 126.1, 111.7, 111.0, 110.2, 101.6, 69.6, 57.0, 27.1, 18.6.

HRMS (ESI) calcd for $C_{16}H_{18}NO_3$ $[M+H]^+$ 272.1287, found 272.1280.

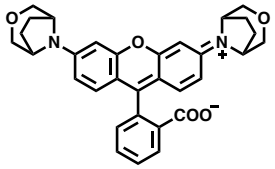


BD 528: S2 is prepared according to a published protocol[1]. A vial was charged with fluorescein ditriflate **S2** (100 mg, 168 μmol, 1.0 eq.), $Pd(OAc)_2$ (7.5 mg, 34 μmol, 0.2 eq.), BINAP (31 mg, 50 μmol, 0.3 eq.), Cs_2CO_3 (153 mg, 469 μmol, 2.8 eq.) and 3-thia-8-azabicyclo [3.2.1] octane 3,3-dioxide hydrochloride (139 mg, 705 μmol, 4.2 eq.). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (2 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 10 h. It was subsequently cooled to room temperature, diluted with MeOH, filtered and concentrated *in vacuo*. Purification of the residue by reverse phase HPLC (eluent, a 30-min linear gradient, from 20% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 530 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 528** (66 mg, 64% yield) as a red solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 7.6 Hz, 1H), 7.71 (td, J = 7.5, 1.2 Hz, 1H), 7.63 (td, J = 7.4, 1.2 Hz, 1H), 7.28 (d, J = 7.6 Hz, 1H), 6.72 (d, J = 8.7 Hz, 2H), 6.55 (d, J = 2.4 Hz, 2H), 6.44 (dd, J = 8.8, 2.4 Hz, 2H), 4.71 – 4.54 (m, 4H), 3.57 – 3.36 (m, 4H), 3.17 – 3.00 (m, 4H), 2.64 – 2.48 (m, 4H), 2.34 – 2.15 (m, 4H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.7, 152.4, 152.1, 145.6, 135.6, 130.1, 129.3, 126.4, 124.6, 124.2, 111.5, 108.5, 101.4, 83.3, 69.8, 54.7, 54.6, 53.0, 52.9, 26.4.

HRMS (ESI) calcd for $C_{32}H_{31}N_2O_7S_2$ $[M+H]^+$ 619.1567, found 619.1573.

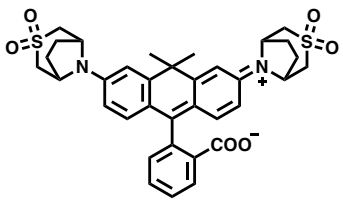


BD 555: S2 is prepared according to a published protocol[1]. A vial was charged with fluorescein ditriflate **S2** (100 mg, 168 μmol, 1.0 eq.), $Pd(OAc)_2$ (7.5 mg, 34 μmol, 0.2 eq.), BINAP (31 mg, 50 μmol, 0.3 eq.), Cs_2CO_3 (153 mg, 469 μmol, 2.8 eq.) and 3-oxa-8-azabicyclo[3.2.1]octane hydrochloride (105 mg, 704 μmol, 4.2 eq.). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (2 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 10 h. It was subsequently cooled to room temperature, diluted with MeOH, filtered and concentrated *in vacuo*. Purification of the residue by reverse phase HPLC (eluent, a 30-min linear gradient, from 20% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 555 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 555** (53 mg, 60% yield) as a purple solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 7.6 Hz, 1H), 7.66 (td, J = 7.5, 1.3 Hz, 1H), 7.60 (td, J = 7.4, 1.1 Hz, 1H), 7.21 (d, J = 7.6 Hz, 1H), 6.64 (d, J = 8.8 Hz, 2H), 6.56 (d, J = 2.4 Hz, 2H), 6.45 (dd, J = 8.8, 2.4 Hz, 2H), 4.15 – 4.02 (m, 4H), 3.93 – 3.81 (m, 4H), 3.59 – 3.45 (m, 4H), 2.19 – 1.89 (m, 8H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.1, 161.2, 159.6, 154.7, 135.3, 133.9, 132.9, 132.6, 132.2, 131.5, 131.5, 116.7, 115.5, 98.9, 72.7, 72.7, 59.2, 27.5.

HRMS (ESI) calcd for $C_{32}H_{31}N_2O_5$ $[M+H]^+$ 523.2227, found 523.2256.

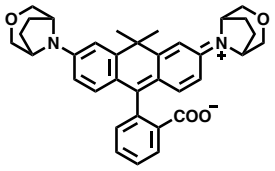


BD 586: S3 is prepared according to a published protocol[1]. A vial was charged with carbofluorescein ditriflate **S3** (100 mg, 161 μmol, 1.0 eq.), $Pd(OAc)_2$ (7.2 mg, 32 μmol, 0.2 equiv), BINAP (30 mg, 48 μmol, 0.3 equiv), Cs_2CO_3 (147 mg, 450 μmol, 2.8 equiv) and 3-thia-8-azabicyclo [3.2.1] octane 3,3-dioxide hydrochloride (133 mg, 676 μmol, 4.2 equiv). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (2 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 10 h. It was subsequently cooled to room temperature, diluted with MeOH, filtered and concentrated *in vacuo*. Purification of the residue by reverse phase HPLC (eluent, a 30-min linear gradient, from 30% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 590 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 586** (69 mg, 66% yield) as an off-white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.6 Hz, 1H), 7.65 (td, *J* = 7.5, 1.2 Hz, 1H), 7.58 (td, *J* = 7.4, 1.2 Hz, 1H), 7.13 (d, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 2.5 Hz, 2H), 6.70 (d, *J* = 8.7 Hz, 2H), 6.55 (dd, *J* = 8.8, 2.4 Hz, 2H), 4.77 – 4.63 (m, 4H), 3.54 – 3.35 (m, 4H), 3.16 – 2.98 (m, 4H), 2.62 – 2.49 (m, 4H), 2.30 – 2.18 (m, 4H), 1.83 (s, 3H), 1.75 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 155.3, 147.1, 143.82, 135.0, 130.3, 129.3, 126.5, 125.1, 124.2, 122.1, 114.0, 111.7, 86.5, 54.7, 54.6, 53.5, 53.4, 38.4, 35.3, 33.5, 27.1, 27.0.

HRMS (ESI) calcd for $C_{35}H_{37}N_2O_6S_2$ $[M+H]^+$ 645.2088, found 645.2048.

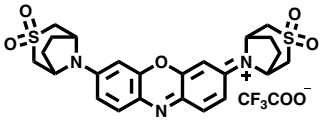


BD 615: S3 is prepared according to a published protocol[1]. A vial was charged with carbofluorescein ditriflate **S3** (100 mg, 161 μmol, 1.0 eq.), $Pd(OAc)_2$ (7.2 mg, 32 μmol, 0.2 equiv), BINAP (30 mg, 48 μmol, 0.3 equiv), Cs_2CO_3 (147 mg, 450 μmol, 2.8 equiv) and 3-oxa-8-azabicyclo[3.2.1]octane hydrochloride (101 mg, 676 μmol, 4.2 equiv). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (2 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 10 h. It was subsequently cooled to room temperature, diluted with MeOH, filtered and concentrated *in vacuo*. Purification of the residue by reverse phase HPLC (eluent, a 30-min linear gradient, from 30% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 620 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 615** (60 mg, 68% yield) as a blue solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (dd, *J* = 7.0, 1.4 Hz, 1H), 7.60 (td, *J* = 7.4, 1.4 Hz, 1H), 7.55 (td, *J* = 7.4, 1.2 Hz, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 2.3 Hz, 2H), 6.60 (d, *J* = 8.7 Hz, 2H), 6.54 (dd, *J* = 8.8, 2.4 Hz, 2H), 4.11 – 4.06 (m, 4H), 3.93 – 3.86 (m, 4H), 3.56 – 3.50 (m, 4H), 2.12 – 1.96 (m, 8H), 1.82 (s, 3H), 1.73 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 155.3, 147.8, 147.2, 134.6, 129.5, 129.0, 127.1, 125.0, 124.0, 121.3, 114.4, 112.3, 87.7, 69.8, 69.7, 57.1, 38.5, 35.6, 32.6, 26.9.

HRMS (ESI) calcd for $C_{35}H_{37}N_2O_4$ $[M+H]^+$ 549.2748, found 549.2761.

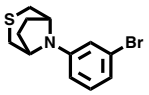


BD 623: S4 is prepared according to a published protocol[1]. A vial was charged with ditriflate **S4** (150 mg, 288 μmol, 1.0 eq.), $Pd(OAc)_2$ (13 mg, 57.6 μmol, 0.2 equiv), BINAP (54 mg, 86.4 μmol, 0.3 equiv), Cs_2CO_3 (263 mg, 806 μmol, 2.8 eq.) and 3-thia-8-azabicyclo [3.2.1] octane 3,3-dioxide hydrochloride (238 mg, 1210 μmol, 4.2 eq.). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (3 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 8 h. It was subsequently cooled to room temperature, diluted with dichloromethane. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (PE/ EA =2/3, v/v) afforded the *N*-acetyl leuco-dye **S5** as a colorless solid (93 mg, 59%). The intermediate **S5** (93 mg, 171 μmol) was taken up in a mixture of dichloromethane (10.0 mL) and water (1.1 mL) and cooled to 0 °C. DDQ (43 mg, 188 μmol) was added, and the reaction was stirred at room temperature for 3 h. A second portion of DDQ (21 mg, 94 μmol) was added, and the reaction was stirred for an additional 0.5 h. The mixture was evaporated, redissolved in CH_3CN, filtered and concentrated to dryness. Purification of the residue by reverse phase HPLC (eluent, a 30-min linear gradient, from 10% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 630 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 623** (41 mg, 48% yield, TFA salt) as a blue solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (d, J = 9.4 Hz, 2H), 7.67 (dd, J = 9.5, 2.4 Hz, 2H), 7.34 (d, J = 2.5 Hz, 2H), 5.38 – 5.34 (m, 4H), 3.65 – 3.57 (m, 4H), 3.53 – 3.45 (m, 4H), 2.53 – 2.40 (m, 4H), 2.29 – 2.21 (m, 4H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 153.5, 149.3, 135.2, 134.7, 119.7, 98.4, 58.2, 55.1, 25.8.

HRMS (ESI) calcd for $C_{24}H_{26}N_3O_5S_2$ $[M]^+$ 500.1308, found 500.1318.

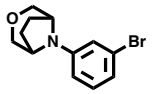


S6:A vial was charged with 1,3-dibromobenzene (7.5 g, 32 mmol, 1.0 eq.), $Pd(OAc)_2$ (1.4 g, 6.4 mmol, 0.2 eq.), BINAP (6.0 g, 9.6 mmol, 0.3 eq.), t-BuONa (6.2 g, 64 mmol, 2.0 eq.) and 3-thia-8-azabicyclo [3.2.1] octane hydrochloride (6.3 g, 38 mmol, 1.2 equiv). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (30 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction mixture was stirred at 100 °C for 6 h. After cooling to room temperature, water (20 mL) was added and CH_2Cl_2 (20 mL × 3) was used to extract organic compounds. The combined organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the residue by silica gel chromatography (PE/EA=50/1, v/v) provided compound **S6** (5.3 g, 58% yield) as a white solid.

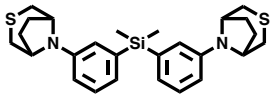
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (t, J = 8.1 Hz, 1H), 6.87 – 6.78 (m, 2H), 6.62 (dd, J = 8.3, 2.4 Hz, 1H), 4.44 – 4.32 (m, 2H), 3.41 – 3.30 (m, 2H), 2.31 – 2.12 (m, 4H), 2.00 – 1.84 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 146.6, 131.1, 124.0, 119.7, 117.6, 113.4, 54.2, 28.8, 27.4.

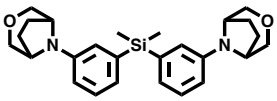
HRMS (ESI) calcd for $C_{12}H_{15}BrNS$ $[M+H]^+$ 284.0103, found 284.0105.



S7:A vial was charged with 1,3-dibromobenzene (7.5 g, 32 mmol, 1.0 eq.), $Pd(OAc)_2$ (1.4 g, 6.4 mmol, 0.2 eq.), BINAP (6.0 g, 9.6 mmol, 0.3 eq.), t-BuONa (6.2 g, 64 mmol, 2.0 eq.) and 3-oxa-8-azabicyclo[3.2.1]octane hydrochloride (5.7 g, 38 mmol, 1.2 equiv). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (30 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction mixture was stirred at 100 °C for 6 h. After cooling to room temperature, water (20 mL) was added and CH_2Cl_2 (20 mL × 3) was used to extract organic compounds. The combined organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the residue by silica gel chromatography (PE/EA=50/1, v/v) provided compound **S7** (5.5 g, 64% yield) as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (t, *J* = 8.1 Hz, 1H), 6.92 – 6.84 (m, 2H), 6.68 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.04 – 3.97 (m, 2H), 3.92 – 3.84 (m, 2H), 3.57 – 3.47 (m, 2H), 2.14 – 1.94 (m, 4H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 148.7, 131.0, 123.8, 120.9, 118.1, 113.9, 69.8, 57.8, 26.9.
HRMS (ESI) calcd for $C_{12}H_{15}BrNO$ $[M+H]^+$ 268.0332, found 268.0338.

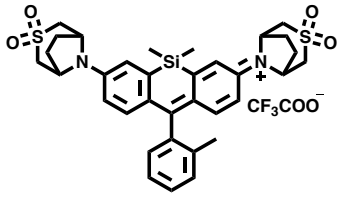


S8:To a flame-dried flask flushed with nitrogen, compound **S6** (3.7 g, 13 mmol, 1.0 equiv) and anhydrous THF (9 mL) were added. The solution was cooled to -78 °C, n-BuLi (8.8 mL of 1.6 M solution in hexane, 14 mmol, 1.1 equiv) was injected with a syringe quickly dropwise, and the mixture was stirred at -78 °C for 20 min. At the same temperature, $SiMe_2Cl_2$ (0.78 mL, 6.5 mmol, 0.5 equiv) was injected with a syringe dropwisely, and the mixture was warmed to room temperature, then stirred for 30 min. It was subsequently quenched with saturated NH_4Cl, diluted with water, and extracted with EtOAc. The organic phase was collected and washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (PE / EA = 20/1, v/v) to afford product **S8** as a white solid (1.3 g, 42%).
^{1}H NMR (400 MHz, CDCl3) δ 7.28 – 7.23 (m, 2H), 7.03 – 6.81 (m, 4H), 6.83 – 6.58 (m, 2H), 4.46 – 4.39 (m, 4H), 3.47 – 3.31 (m, 4H), 2.30 – 2.12 (m, 8H), 1.98 – 1.77 (m, 4H), 0.52 (s, 6H).
^{13}C NMR (101 MHz, CDCl3) δ 144.6, 139.8, 129.3, 123.0, 120.8, 116.0, 53.9, 28.8, 27.0,-2.1.
HRMS (ESI) calcd for $C_{26}H_{35}N_2S_2Si$ [M+H]+ 467.2005, found 467.2019.



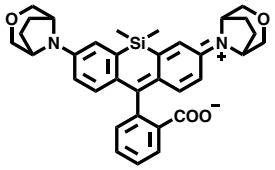
S9:To a flame-dried flask flushed with nitrogen, compound **S7** (3.5 g, 13 mmol, 1.0 equiv) and anhydrous THF (9 mL) were added. The solution was cooled to -78 °C, n-BuLi (8.8 mL of 1.6 M solution in hexane, 14 mmol, 1.1 equiv) was injected with a syringe quickly dropwise, and the mixture was stirred at -78 °C for 20 min. At the same temperature, $SiMe_2Cl_2$ (0.78 mL, 6.5 mmol, 0.5 equiv) was injected with a syringe dropwisely, and the mixture was warmed to room temperature, then stirred for 30 min. It was subsequently quenched with saturated NH_4Cl, diluted with water, and extracted with EtOAc. The organic phase was collected and washed with brine,

dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (PE / EA = 20/1, v/v) to afford product **S9** as a white solid (1.3 g, 42%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.22 (m, 2H), 7.01 – 6.90 (m, 4H), 6.88 – 6.77 (m, 2H), 4.09 – 3.98 (m, 4H), 3.98 – 3.83 (m, 4H), 3.56 – 3.42 (m, 4H), 2.10 – 2.01 (m, 4H), 1.99 – 1.91 (m, 4H), 0.51 (s, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 146.8, 139.6, 129.1, 124.4, 121.2,116.4, 70.0, 57.3, 26.7,-2.1.
HRMS (ESI) calcd for $C_{26}H_{35}N_2S_2Si$ $[M+H]^+$ 435.2462, found 435.2477.



BD 634: Compound **S8** (150 mg, 0.32 mmol, 1.0 eq.), *o*-tolualdehyde (190 mg, 1.6 mmol, 5.0 eq.) and *p*-TsOH·H_2O (61 mg, 0.32 mmol, 1.0 eq.) were mixed in a sealable pressure tube. The tube was sealed tightly and heated at 140 °C for 8 h. After cooling to room temperature, the mixture was diluted with MeOH (3 mL), then added chloranil (79 mg, 0.32 mmol, 1.0 eq.) and stirred for 2 h. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH=15/1, v/v, with constant 1% v/v AcOH additive) to afford intermediate **S10** as blue-green solid (39 mg, 16%).

The intermediate **S10** (39 mg, 69 mmol) was taken up in MeOH (5 mL) and added $NaBH_4$ (5.2 mg, 140 mmol). The reaction mixture was stirred for 0.5 h at 50°C. After cooling to room temperature, the mixture was diluted with H_2O and then extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4, concentrated *in vacuo* and directly used for the next reaction. The residue was dissolved in CH_2Cl_2 (2 mL) and a solution of 3-chloroperozybenzoic acid (126 mg, 550 mmol) in CH_2Cl_2 (2 mL) was added dropwisely at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, then allowed to warm to room temperature for an additional 2h. After that, the mixture was washed with aq. $NaHSO_3$ and extracted with CH_2Cl_2. The collected organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was again dissolved in CH_2Cl_2 (2 mL), and DDQ (15 mg, 69 mmol) was added in one portion. The mixture was stirred for 2 h, and then washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was redissolved in MeOH (1 mL), and AcOH (10 μL) was added (immediate blue color). After stirring the blue solution at room temperature for 10 min, the mixture was concentrated to dryness and purified by HPLC (eluent, a 30-min linear gradient, from 10% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 630 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 634** (16 mg, 38% yield, TFA salt) as a blue solid.
^{1}H NMR (400 MHz, DMF-d_7) δ 7.96 (d, J = 2.5 Hz, 2H), 7.61 – 7.45 (m, 3H), 7.30 (dd, J = 7.6, 1.3 Hz, 1H), 7.25 – 7.15 (m, 4H), 5.65 – 5.30 (m, 4H), 3.67 – 3.53 (m, 8H), 2.61 – 2.52 (m, 4H), 2.37 – 2.27 (m, 4H), 2.10 (s, 3H), 0.70 (s, 3H), 0.69 (s, 3H).
^{13}C NMR (101 MHz, CD_3CN) δ 173.5, 151.5, 151.1, 143.8, 139.2, 136.6, 131.2, 130.2, 130.0, 129.7, 126.6, 123.5, 116.8, 59.0, 55.3, 26.8, 19.5, -1.4, -1.5.
HRMS (ESI) calcd for $C_{34}H_{39}N_2O_4S_2Si$ $[M]^+$ 631.2115, found 631.2107.



BD 655: Compound **S9** (139 mg, 0.32 mmol, 1.0 eq.), 2-formylbenzoic acid (240 mg, 1.6 mmol, 5.0 eq.) and $CuBr_2$ (7 mg, 0.03 mmol, 0.1 eq.) were mixed in a sealable pressure tube. The tube was sealed tightly and heated at 140 °C for 4 h. After cooling to room temperature, the reaction mixture was dissolved in MeOH (5 mL), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (PE / EA = 5/2, v/v) to afford product **BD 655** as a blue-green solid (72 mg, 40%).

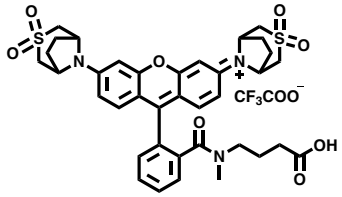
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.6 Hz, 1H), 7.66 (td, *J* = 7.5, 1.2 Hz, 1H), 7.56 (td, *J* = 7.5, 1.0 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 2.7 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.59 (dd, *J* = 8.9, 2.8 Hz, 2H), 4.11 – 4.05 (m, 4H), 3.91 – 3.84 (m, 4H), 3.56 – 3.46 (m, 4H), 2.12 – 1.99 (m, 4H), 2.02 – 1.93 (m, 4H), 0.61 (s, 3H), 0.60 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 154.0, 146.5, 137.7, 133.8, 133.7, 129.0, 128.7, 127.1, 125.9, 124.8, 119.9, 116.2, 91.6, 69.9, 69.8, 57.0, 56.9, 26.9, 26.9, 0.5, -1.5.

HRMS (ESI) calcd for $C_{34}H_{37}N_2O_4Si$ $[M+H]^+$ 565.2517, found 565.2528.

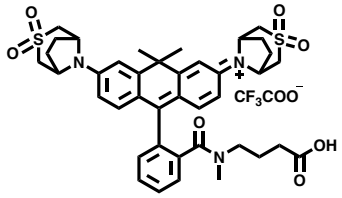
General Method A: Functionalization with carboxylic acid

Representative Procedure for **BD Yellow**:

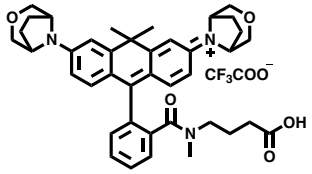


BD Yellow: To a flame-dried flask flushed with nitrogen, **BD528** (24 mg, 0.038 mmol, 1.0 eq.) and anhydrous 1,2-dichloroethane (2 mL) were added. Then $POCl_3$ (0.18 mL, 1.9 mmol, 50.0 eq.) was added by a syringe. The reaction was stirred at 75 °C for 2 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. CH_2Cl_2 (2 mL) was added and removed by rotary evaporation, and the mixture was placed under high vacuum (25 mmHg) for 0.5 h. To the round-bottomed flask, anhydrous CH_2Cl_2 (1 mL) was added by a syringe and the reaction was placed in an ice bath (–15 °C). Meanwhile, methyl 4-(methylamino)butanoate hydrochloride (63 mg, 0.38 mmol, 10.0 eq.) and triethylamine (0.05 mL) was dissolved in CH_2Cl_2 (1 mL), giving amine solution. After the reaction mixture had stirred for 10 minutes in the external bath, the free-based amine was added via syringe in one addition. The reaction was stirred at the same temperature for another 20 minutes and then allowed to warm to 25 °C and stirred for an additional 20 minutes. 1 N HCl (2 mL) was added by syringe and the two layers separated. The aqueous layer was further extracted with CH_2Cl_2 (2 x 3 mL). The organic layers were combined, washed with brine and dried over Na_2SO_4. The solvent was removed by rotary evaporation and the crude methyl ester was taken directly to the next reaction. The crude methyl ester **24** (0.038 mmol) was added into a 25 mL flask. THF (5 mL) was added by a syringe followed by ddH_2O (2 mL). The reaction was stirred at 0 °C for 10 min. A solution of 1 N NaOH (0.19 mL, 0.19 mmol, 5.0 eq.) was added and the reaction was stirred at this same temperature for 2 h before acidified with glacial AcOH (0.5 mL, 8.75 mmol, 10.0 eq). The reaction was concentrated *in vacuo*. Purification of the residue by HPLC (eluent, a 30-min linear gradient, from 10% to 90% solvent B; flow rate, 5.0 mL/min;

detection wavelength, 540 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD Yellow** (19 mg, 72% yield) as a red solid.
^{1}H NMR (400 MHz, CD_3CN) δ 7.77 – 7.60 (m, 3H), 7.51 – 7.29 (m, 3H), 7.18 – 7.03 (m, 4H), 5.07 – 5.01 (m, 4H), 3.75 – 3.30 (m, 8H), 3.24 – 3.13 (m, 2H), 2.91 (s, 3H), 2.58 – 2.48 (m, 4H), 2.37 – 2.16 (m, 4H), 1.78 – 1.69 (m, 2H), 1.38 – 1.25 (m, 2H).
^{13}C NMR (101 MHz, CD_3CN) δ 174.6, 169.0, 159.3, 154.1, 137.7, 134.4, 134.0, 131.1, 131.0, 130.5, 128.4, 126.7, 116.7, 116.3, 99.7, 58.8, 55.6, 55.6, 46.7, 37.7, 31.0, 27.1, 22.3.
HRMS (ESI) calcd for $C_{37}H_{40}N_3O_8S_2$ $[M]^+$ 718.2251, found 718.2253.



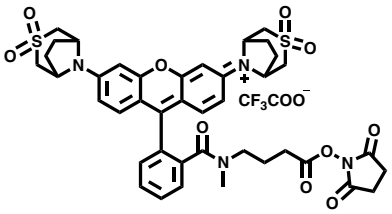
BD Orange: The title compound (25 mg, 78%, purple solid) was prepared from **BD 586** according to general method A.
^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.72 – 7.65 (m, 2H), 7.63 – 7.58 (m, 1H), 7.46 – 7.39 (m, 1H), 7.32 – 7.23 (m, 4H), 6.90 (dd, J = 9.3, 2.4 Hz, 2H), 5.11 (s, 4H), 3.49 – 3.30 (m, 8H), 3.24 – 3.16 (m, 2H), 2.92 (s, 3H), 2.58 – 2.45 (m, 4H), 2.30 – 2.22 (m, 4H), 1.83 (s, 3H), 1.81 – 1.73 (m, 2H), 1.71 (s, 3H), 1.41 – 1.30 (m, 2H).
^{13}C NMR (101 MHz, CD_3CN) δ 174.6, 169.3, 167.3, 159.7, 153.1, 140.1, 137.3, 135.1, 131.1, 130.3, 130.1, 128.1, 123.2, 115.2, 113.8, 59.1, 59.0, 55.4, 46.9, 43.1, 37.7, 34.8, 32.4, 31.2, 27.0, 26.9, 22.5.
HRMS (ESI) calcd for $C_{40}H_{46}N_3O_7S_2$ $[M]^+$ 744.2772, found 744.2790.



BD Red: The title compound (28 mg, 80%, blue solid) was prepared from **BD 615** according to general method A.
^{1}H NMR (400 MHz, CD_3CN) δ 7.67 – 7.62 (m, 2H), 7.59 – 7.49 (m, 1H), 7.42 – 7.34 (m, 1H), 7.17 – 7.14 (m, 2H), 7.14 – 7.08 (m, 2H), 6.79 – 6.72 (m, 2H), 4.65 – 4.62 (m, 4H), 3.72 – 3.65 (m, 8H), 3.26 – 3.14 (m, 2H), 2.88 (s, 3H), 2.18 – 2.10 (m, 4H), 2.12 – 2.04 (m, 4H), 1.83 – 1.78 (m, 2H), 1.77 (s, 3H), 1.66 (s, 3H), 1.45 – 1.37 (m, 2H).
^{13}C NMR (101 MHz, CD_3CN) δ 174.4, 169.5, 163.0, 158.2, 152.8, 139.1, 137.3, 135.4, 131.2, 130.0, 129.9, 127.9, 121.9, 114.6, 113.4, 72.8, 58.8, 58.7, 46.9, 42.6, 37.8, 31.4, 27.1, 27.0, 22.6.
HRMS (ESI) calcd for $C_{40}H_{46}N_3O_5$ $[M]^+$ 648.3432, found 648.3440.

General Method B: Conversion of carboxylic acids to NHS esters

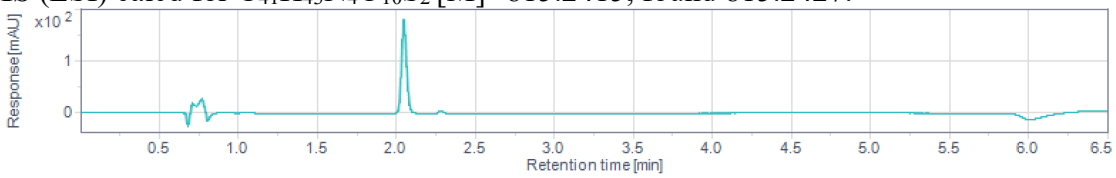
Representative Procedure for **BD Yellow$_{NHS}$**:

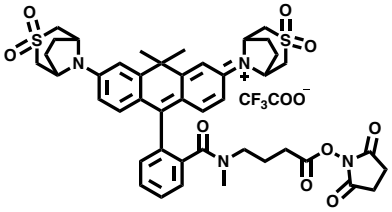


BD Yellow (16) (10 mg, 14 µmol, 1.0 eq.), TSTU (8.6 mg, 28 µmol, 2.0 eq.) and 2,6-Lutidine (15 mg, 140 µmol, 10.0 eq.) were dissolved in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 4 h. The product was purified by RP-HPLC (eluent, a 20-min linear gradient, from 20% to 80% solvent B; flow rate, 5.0 mL/min; detection wavelength, 540 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) and freeze-dried to yield **BD Yellow$_{NHS}$** (8 mg, 80% yield) as a red solid.

Analytical HPLC: 97.6% purity (4.6 mm × 150 mm 5 µm C18 column; 2 µL injection; 5-100% CH_3CN/H_2O, linear-gradient, with constant 0.1% v/v TFA additive; 6 min run; 0.6 mL/min flow; ESI; positive ion mode; detection at 600 nm).

HRMS (ESI) calcd for $C_{41}H_{43}N_4O_{10}S_2$ $[M]^+$ 815.2415, found 815.2427.

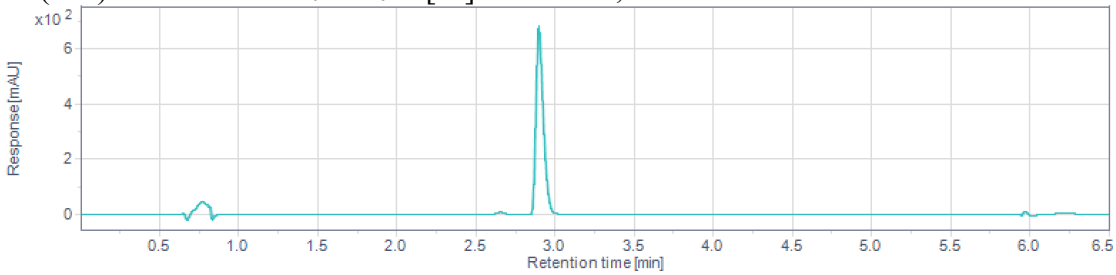


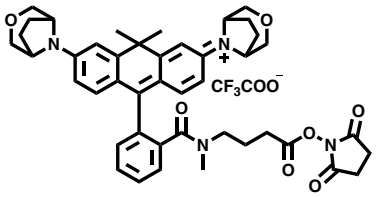


BD Orange$_{NHS}$: The title compound (8.8 mg, 76%, purple solid) was prepared from **BD Orange (17)** according to general method B.

Analytical HPLC: 98.4% purity (4.6 mm × 150 mm 5 µm C18 column; 2 µL injection; 5-100% CH_3CN/H_2O, linear-gradient, with constant 0.1% v/v TFA additive; 6 min run; 0.6 mL/min flow; ESI; positive ion mode; detection at 600 nm).

HRMS (ESI) calcd for $C_{44}H_{49}N_4O_9S_2$ $[M]^+$ 841.2935, found 841.2962.

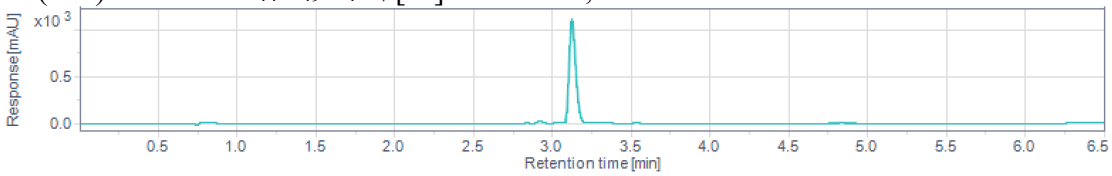


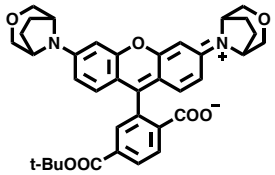


BD Red$_{NHS}$: The title compound (9.0 mg, 78%, blue solid) was prepared from **BD Red** according to general method B.

Analytical HPLC: 96.8% purity (4.6 mm × 150 mm 5 μm C18 column; 2 μL injection; 5-100% CH_3CN/H_2O, linear-gradient, with constant 0.1% v/v TFA additive; 6 min run; 0.6 mL/min flow; ESI; positive ion mode; detection at 630 nm).

HRMS (ESI) calcd for $C_{44}H_{49}N_4O_7$ $[M]^+$ 745.3596, found 745.3582.



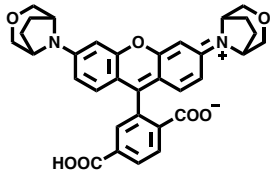


BD 566$_{COOt\text{-}Bu}$: S11 is prepared according to a published protocol[1]. A vial was charged with ditriflate **S11** (100 mg, 144 μmol, 1.0 eq.), $Pd(OAc)_2$ (6.4 mg, 29 μmol, 0.2 eq.), BINAP (27 mg, 43 μmol, 0.3 eq.), Cs_2CO_3 (131 mg, 403 μmol, 2.8 eq.) and 3-oxa-8-azabicyclo[3.2.1]octane hydrochloride (90 mg, 605 μmol, 4.2 eq.). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (2 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 10 h. It was subsequently cooled to room temperature, diluted with MeOH, filtered and concentrated *in vacuo*. Purification of the residue by silica gel chromatography (CH_2Cl_2/MeOH (2M NH_3)=20/1, v/v) provided **BD 566$_{COOt\text{-}Bu}$** (48 mg, 54% yield) as a purple solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (dd, J = 8.0, 1.4 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.80 – 7.75 (m, 1H), 6.63 – 6.53 (m, 4H), 6.48 – 6.41 (m, 2H), 4.09 – 4.05 (m, 4H), 3.92 – 3.84 (m, 5H), 3.56 – 3.49 (m, 4H), 2.16 – 1.96 (m, 8H), 1.55 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 164.5, 153.3, 149.2, 138.0, 130.8, 130.7, 129.6, 125.4, 125.4, 125.0, 124.9, 111.7, 107.9, 101.7, 82.5, 69.7, 69.6, 57.0, 56.9, 28.2, 27.0.

HRMS (ESI) calcd for $C_{37}H_{39}N_2O_7$ $[M+H]^+$ 623.2752, found 623.2764.



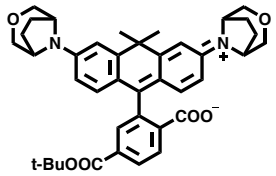
BD 566$_{COOH}$: **BD 566$_{COOt\text{-}Bu}$** (48 mg, 77 mmol) was dissolved in CH_2Cl_2 (1.5 mL), and trifluoroacetic acid (0.3 mL) was added. The reaction was stirred at room temperature for 2 h and

then concentrated *in vacuo*. Purification of the residue by reverse phase HPLC (eluent, a 30-min linear gradient, from 30% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 560 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 566$_{COOH}$** (41 mg, 95% yield) as a purple solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.46 – 8.36 (m, 2H), 8.02 – 7.97 (m, 1H), 7.17 – 7.05 (m, 6H), 4.67 – 4.61 (m, 4H), 3.81 – 3.68 (m, 8H), 2.26 – 2.09 (m, 8H).

^{13}C NMR (101 MHz, CD_3OD) δ 167.7, 167.4, 159.7, 159.7, 154.7, 136.0, 135.9, 135.4, 132.9, 132.7, 132.4, 132.3, 116.9, 115.4, 99.0, 72.8, 59.3, 27.5.

HRMS (ESI) calcd for $C_{33}H_{31}N_2O_7$ $[M+H]^+$ 567.2126, found 567.2148.

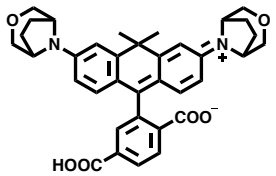


BD 626$_{COOt\text{-}Bu}$: S12 is prepared according to a published protocol[1]. A vial was charged with ditriflate **S12** (104 mg, 144 μmol, 1.0 eq.), $Pd(OAc)_2$ (6.4 mg, 29 μmol, 0.2 eq.), BINAP (27 mg, 43 μmol, 0.3 eq.), Cs_2CO_3 (131 mg, 403 μmol, 2.8 eq.) and 3-oxa-8-azabicyclo[3.2.1]octane hydrochloride (90 mg, 605 μmol, 4.2 eq.). The vial was sealed and evacuated/backfilled with N_2 for three times. Toluene (2 mL) was added, and the reaction was flushed again with N_2 for three times. The reaction was stirred at 100 °C for 6 h. It was subsequently cooled to room temperature, diluted with MeOH, filtered and concentrated *in vacuo*. Purification of the residue by silica gel chromatography (CH_2Cl_2/MeOH=40/1, v/v) provided **BD 626$_{COOt\text{-}Bu}$** (42 mg, 45% yield) as a blue solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (dd, *J* = 8.0, 1.4 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.66 – 7.61 (m, 1H), 6.96 (d, *J* = 2.2 Hz, 2H), 6.60 – 6.51 (m, 4H), 4.14 – 4.03 (m, 4H), 3.95 – 3.83 (m, 4H), 3.58 – 3.47 (m, 4H), 2.13 – 1.95 (m, 8H), 1.82 (s, 3H), 1.73 (s, 3H), 1.54 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 164.5, 155.5, 147.9, 147.1, 137.9, 130.2, 130.1, 129.5, 125.1, 124.9, 120.6, 114.5, 112.4, 88.0, 82.5, 69.9, 69.8, 57.1, 57.0, 38.5, 35.4, 33.1, 28.2, 26.9.

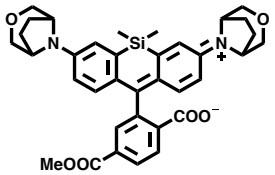
HRMS (ESI) calcd for $C_{40}H_{45}N_2O_6$ $[M+H]^+$ 649.3272, found 649.3291.



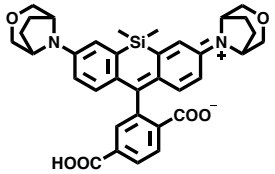
BD 626$_{COOH}$:**BD 626$_{COOt\text{-}Bu}$** (42 mg, 65 mmol) was dissolved in CH_2Cl_2 (1.5 mL), and trifluoroacetic acid (0.3 mL) was added. The reaction was stirred at room temperature for 2 h and then concentrated *in vacuo*. Purification of the residue by reverse phase HPLC (eluent, a 30-min linear gradient, from 30% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 630 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 626$_{COOH}$** (35 mg, 92% yield) as a blue solid.

^{1}H NMR (400 MHz, DMF-d_7) δ 8.31 (dd, *J* = 8.0, 1.4 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 7.72 – 7.66 (m, 1H), 7.36 – 7.28 (m, 2H), 6.83 (dd, *J* = 8.9, 2.4 Hz, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 4.50 – 4.27 (m, 4H), 3.83 – 3.74 (m, 4H), 3.58 – 3.46 (m, 4H), 2.07 – 1.94 (m, 8H), 1.92 (s, 3H), 1.80 (s, 3H).

Analytical HPLC: >99% purity (4.6 mm × 150 mm 5 μm C18 column; 2 μL injection; 5-100% CH_3CN/H_2O, linear-gradient, with constant 0.1% v/v TFA additive; 6 min run; 0.6 mL/min flow; ESI; positive ion mode; detection at 630 nm).
HRMS (ESI) calcd for $C_{36}H_{37}N_2O_6$ $[M+H]^+$ 593.2646, found 593.2641.



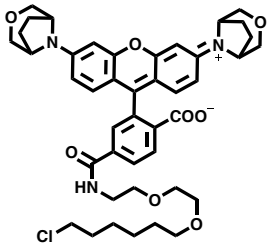
BD 666$_{COOMe}$: Compound **S9** (130 mg, 0.30 mmol, 1.0 eq.), 2-formyl-4-(methoxycarbonyl) benzoic acid (312 mg, 1.5 mmol, 5.0 eq.) and $CuBr_2$ (7 mg, 0.03 mmol, 0.1 eq.) were mixed in a sealable pressure tube. The tube was sealed tightly and heated at 140 °C for 4 h. After cooling to room temperature, the reaction mixture was dissolved in MeOH (5 mL), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (PE / EA = 10/1, v/v) to afford product **BD 666$_{COOMe}$** as a blue-green solid (97 mg, 52%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 8.0, 1.3 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.99 – 7.96 (m, 1H), 7.04 (d, *J* = 2.7 Hz, 2H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.61 (dd, *J* = 8.9, 2.8 Hz, 2H), 4.10 – 4.05 (m, 4H), 3.91 (s, 3H), 3.90 – 3.84 (m, 4H), 3.56 – 3.49 (m, 4H), 2.14 – 1.93 (m, 8H), 0.65 (s, 3H), 0.59 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 166.0, 154.6, 146.5, 137.4, 135.2, 133.0, 130.2, 130.1, 128.4, 126.0, 125.9, 120.0, 116.4, 91.7, 69.8, 69.7, 57.0, 52.8, 26.9, 26.9, 0.4, -1.2.
HRMS (ESI) calcd for $C_{36}H_{39}N_2O_6Si$ $[M+H]^+$ 623.2572, found 623.2560.



BD 666$_{COOH}$:To a solution of **BD666$_{COOMe}$** (97 mg, 0.16 mmol, 1.0 eq.) in THF/ddH_2O (2:1, 2 mL in total) was added 1 N LiOH (800 μL, 0.80 mmol, 5.0 eq). The reaction was stirred at room temperature for 1 h. It was subsequently acidified with 1 N HCl (900 μL), diluted with water, and extracted with CH_2Cl_2 (3×). The organic extracts were combined and dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2 / MeOH = 25/1, v/v) to afford product **BD 666$_{COOH}$** as a blue-green solid (78 mg, 82%).
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (dd, *J* = 7.9, 1.3 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.79 – 7.75 (m, 1H), 7.21 (d, *J* = 2.7 Hz, 2H), 6.77 (dd, *J* = 8.9, 2.6 Hz, 2H), 6.68 (d, *J* = 8.8 Hz, 2H), 4.37 – 4.08 (m, 4H), 3.69 – 3.61 (m, 4H), 3.47 – 3.39 (m, 4H), 1.98 – 1.80 (m, 8H), 0.64 (s, 3H), 0.54 (s, 3H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 169.0, 166.1, 158.4, 158.1, 146.4, 136.4, 131.5, 130.2, 128.6, 127.6, 126.1, 124.7, 119.9, 116.4, 91.1, 68.9, 55.8, 26.4, 26.4, -0.1, -1.3.
HRMS (ESI) calcd for $C_{35}H_{37}N_2O_6Si$ $[M+H]^+$ 609.2415, found 609.2420.

General Method C: Functionalization with HaloTag ligand

Representative Procedure for **BD 566$_{HTL}$**:

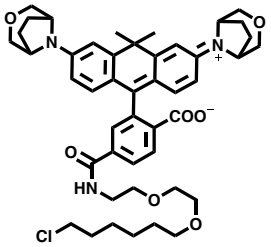


BD 566$_{COOH}$ (22 mg, 40 μmol, 1.0 eq.), HaloTag(O2)amine (13 mg, 60 μmol, 1.5 eq.) and BOP (21 mg, 48 μmol, 1.2 eq.) were dissolved in anhydrous DMF (2 mL). Then DIPEA (13 μL, 80 μmol, 2.0 eq.) was added and the mixture was stirred at room temperature overnight. Purification of the mixture by reverse phase HPLC (eluent, a 30-min linear gradient, from 30% to 95% solvent B; flow rate, 5.0 mL/min; detection wavelength, 560 nm; eluent A (ddH_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN)) provided **BD 566$_{HTL}$** (30 mg, 84% yield) as a purple solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (d, *J* = 8.3 Hz, 1H), 8.22 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.84 (d, *J* = 1.8 Hz, 1H), 7.19 – 7.05 (m, 6H), 4.68 – 4.59 (m, 4H), 3.80 – 3.70 (m, 8H), 3.70 – 3.53 (m, 8H), 3.52 (t, *J* = 6.6 Hz, 2H), 3.44 (t, *J* = 6.5 Hz, 2H), 2.28 – 2.05 (m, 8H), 1.76 – 1.67 (m, 2H), 1.55 – 1.46 (m, 2H), 1.44 – 1.27 (m, 4H).

Analytical HPLC: 98.6% purity (4.6 mm × 150 mm 5 μm C18 column; 2 μL injection; 5-100% CH_3CN/H_2O, linear-gradient, with constant 0.1% v/v TFA additive; 6 min run; 0.6 mL/min flow; ESI; positive ion mode; detection at 560 nm).

HRMS (ESI) calcd for $C_{43}H_{51}ClN_3O_8$ $[M+H]^+$ 772.3359, found 772.3383.

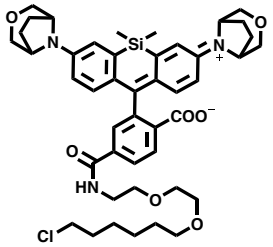


BD 626$_{HTL}$: The title compound (28 mg, 87%, blue solid) was prepared from **BD 626$_{COOH}$** according to general method C.

^{1}H NMR (400 MHz, CD_3CN) δ 8.27 (d, *J* = 8.2 Hz, 1H), 8.06 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.66 (d, *J* = 1.7 Hz, 1H), 7.31 (t, *J* = 5.4 Hz, 1H), 7.18 (d, *J* = 2.4 Hz, 2H), 6.93 (d, *J* = 9.3 Hz, 2H), 6.71 (dd, *J* = 9.3, 2.4 Hz, 2H), 4.61 – 4.54 (m, 4H), 3.76 – 3.63 (m, 8H), 3.63 – 3.46 (m, 10H), 3.35 (t, *J* = 6.5 Hz, 2H), 2.17 – 2.01 (m, 8H), 1.82 (s, 3H), 1.72 (s, 3H), 1.70 – 1.65 (m, 2H), 1.51 – 1.20 (m, 6H).

Analytical HPLC: >99% purity (4.6 mm × 150 mm 5 μm C18 column; 2 μL injection; 5-100% CH_3CN/H_2O, linear-gradient, with constant 0.1% v/v TFA additive; 6 min run; 0.6 mL/min flow; ESI; positive ion mode; detection at 620 nm).

HRMS (ESI) calcd for $C_{46}H_{57}ClN_3O_7$ $[M+H]^+$ 798.3880, found 798.3872.



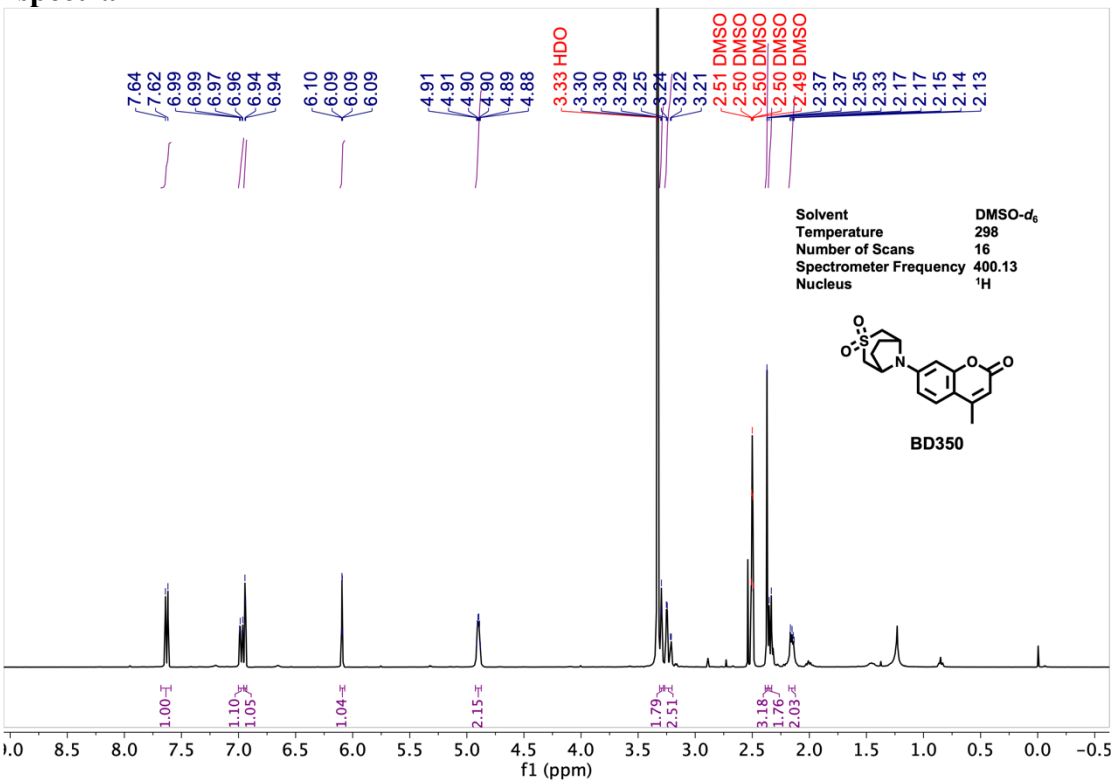
BD 666$_{HTL}$: The title compound (25 mg, 78%, blue-green solid) was prepared from **BD 666$_{COOH}$** according to general method C.

^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (d, *J* = 8.2 Hz, 1H), 8.13 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.74 (d, *J* = 1.7 Hz, 1H), 7.39 (d, *J* = 2.7 Hz, 2H), 6.95 (d, *J* = 9.4 Hz, 2H), 6.78 (dd, *J* = 9.5, 2.7 Hz, 2H), 4.75 – 4.54 (m, 4H), 3.79 – 3.55 (m, 16H), 3.51 (t, *J* = 6.6 Hz, 2H), 3.43 (t, *J* = 6.5 Hz, 2H), 2.26 – 2.01 (m, 8H), 1.76 – 1.65 (m, 2H), 1.51 (p, *J* = 6.8 Hz, 2H), 1.46 – 1.24 (m, 2H), 0.64 (s, 3H), 0.59 (s, 3H).

Analytical HPLC: 98.8% purity (4.6 mm × 150 mm 5 µm C18 column; 2 µL injection; 5-100% CH_3CN/H_2O, linear-gradient, with constant 0.1% v/v TFA additive; 6 min run; 0.6 mL/min flow; ESI; positive ion mode; detection at 660 nm).

HRMS (ESI) calcd for $C_{45}H_{57}ClN_3O_7Si$ $[M+H]^+$ 814.3649 found 814.3650.

NMR spectra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6.09
6.09
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4.91
4.90
4.90
4.89
4.88
3.33 HDO
3.30
3.30
3.29
3.25
3.24
3.22
3.21
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.37
2.37
2.35
2.33
2.17
2.17
2.15
2.14
2.13
Solvent DMSO-d6
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
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1.79
2.51
3.18
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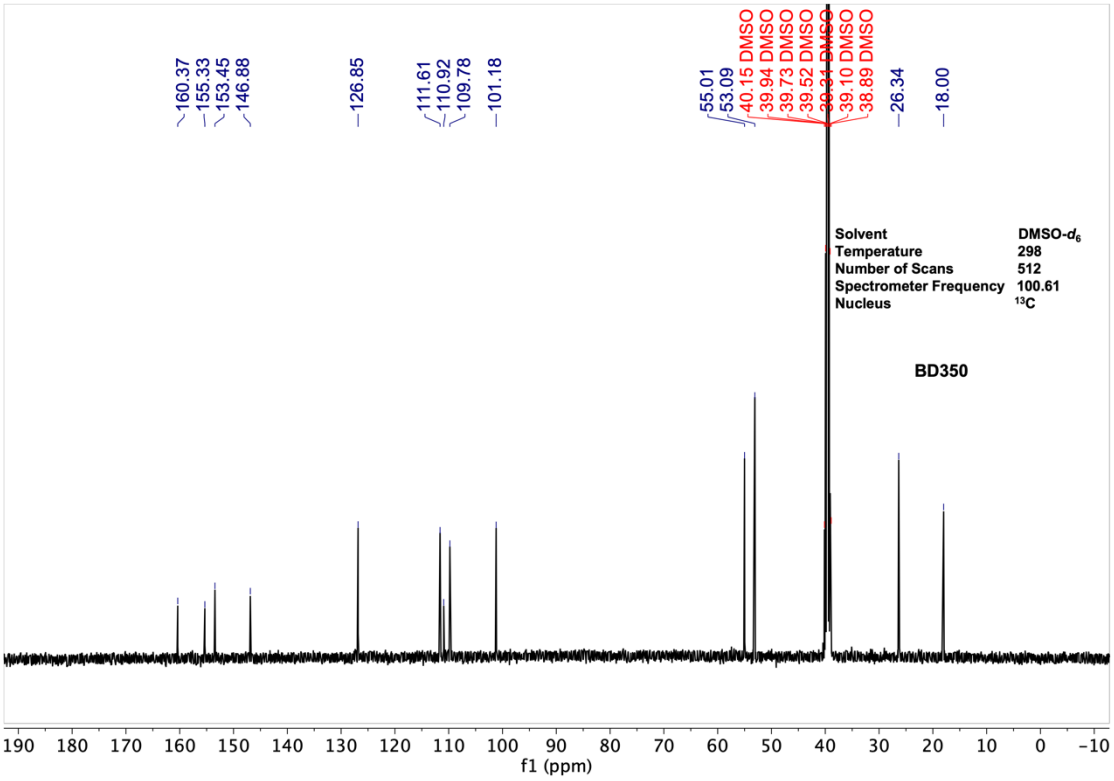
2.03
f1 (ppm)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53.09
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39.73 DMSO
39.52 DMSO
39.31 DMSO
39.10 DMSO
38.89 DMSO
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Solvent DMSO-d6
Temperature 298
Number of Scans 512
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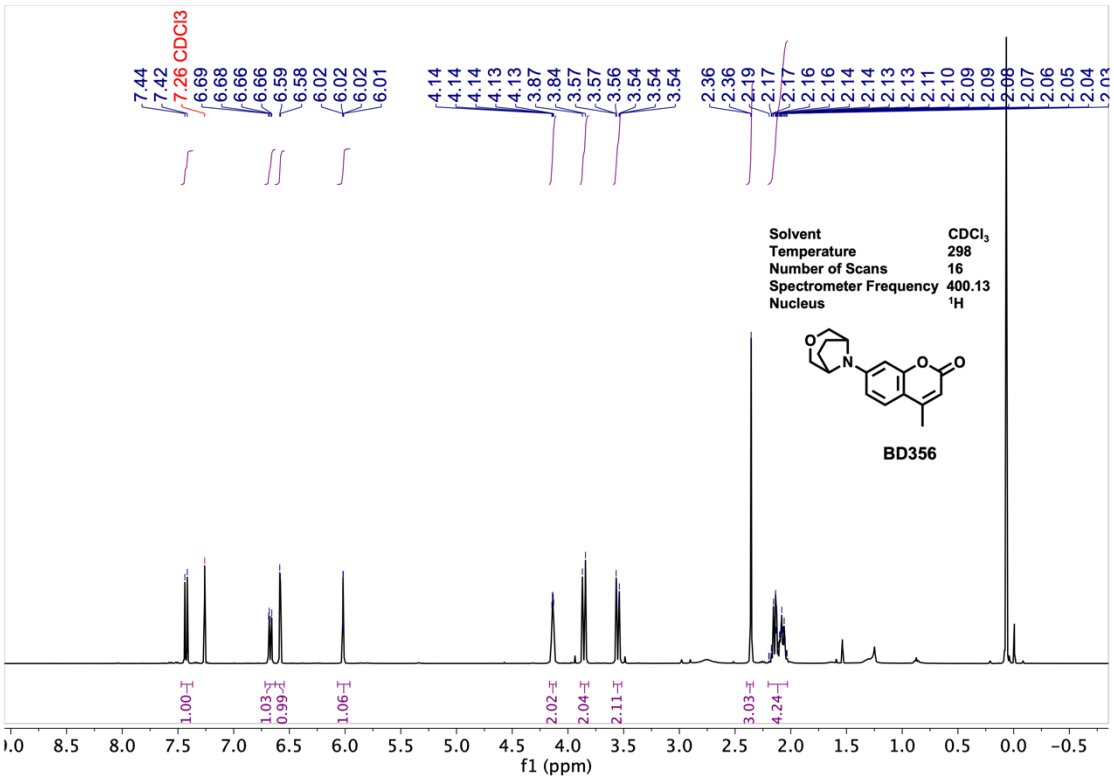
Nucleus 13C
BD350
f1 (ppm)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6.01
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4.14
4.14
4.13
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3.56
3.54
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3.54
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2.19
2.17
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2.14
2.14
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2.05
2.04
Solvent CDCl3
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Number of Scans 16
Spectrometer Frequency 400.13
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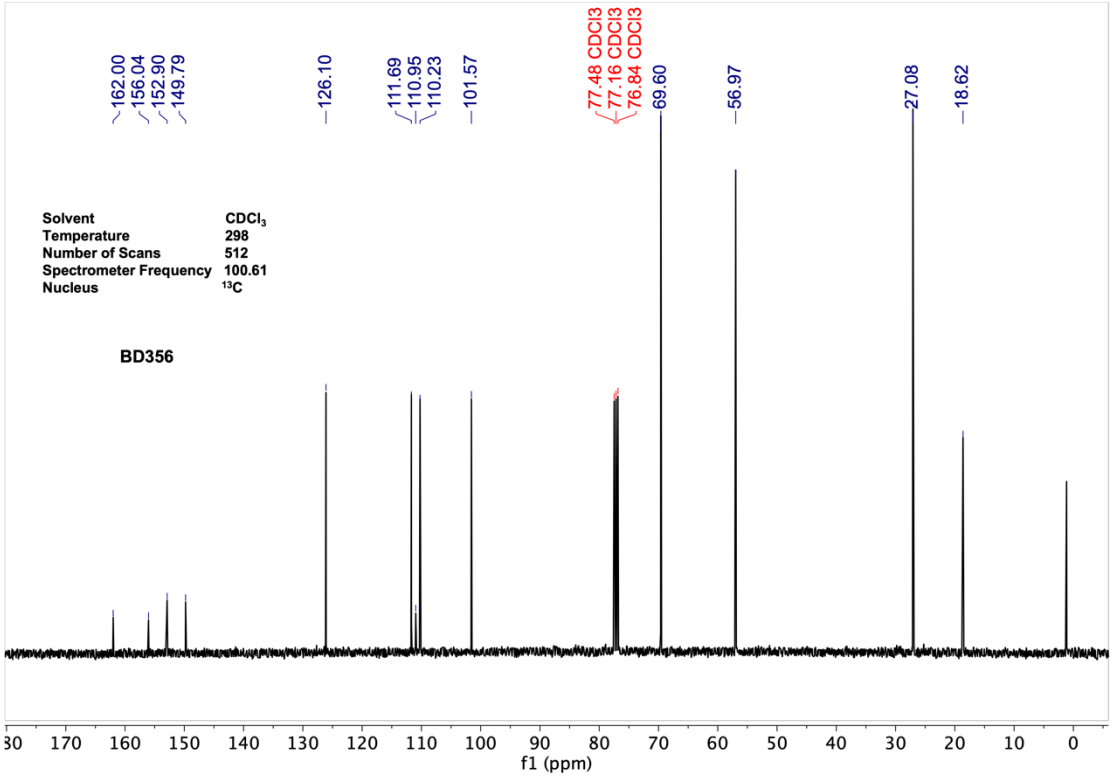
f1 (ppm)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110.95
110.23
101.57
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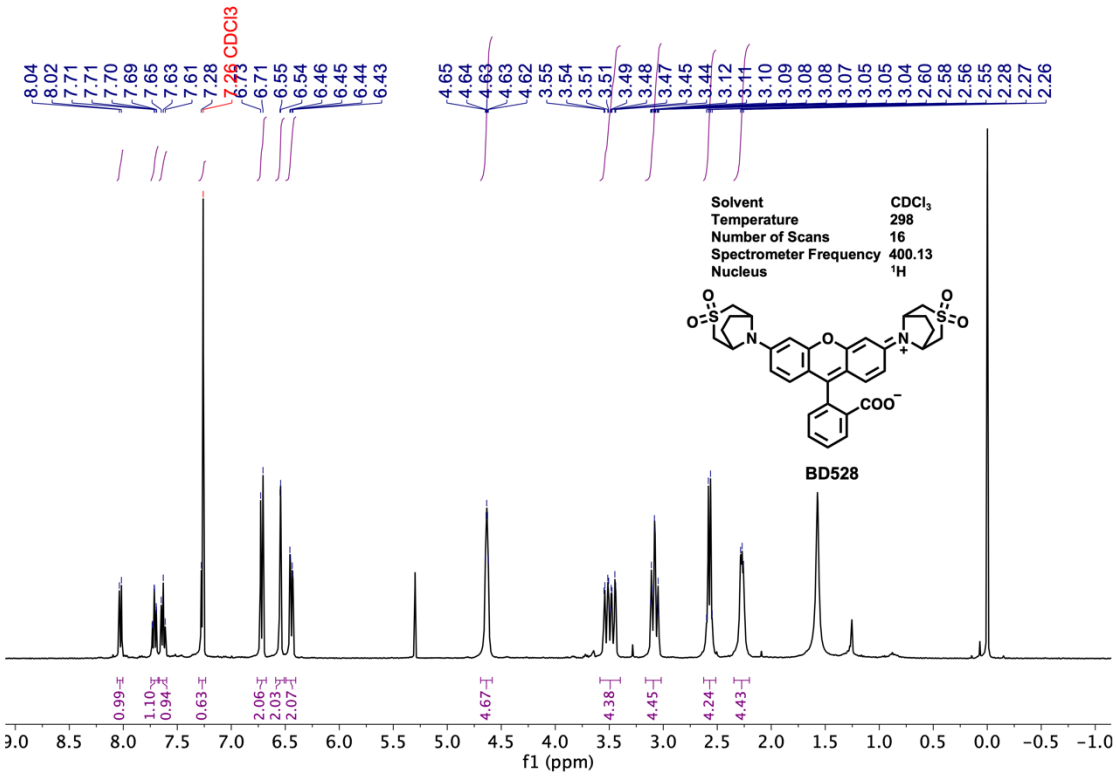
77.16 CDCl3
76.84 CDCl3
69.60
56.97
27.08
18.62
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
BD356
f1 (ppm)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2.27
2.26
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
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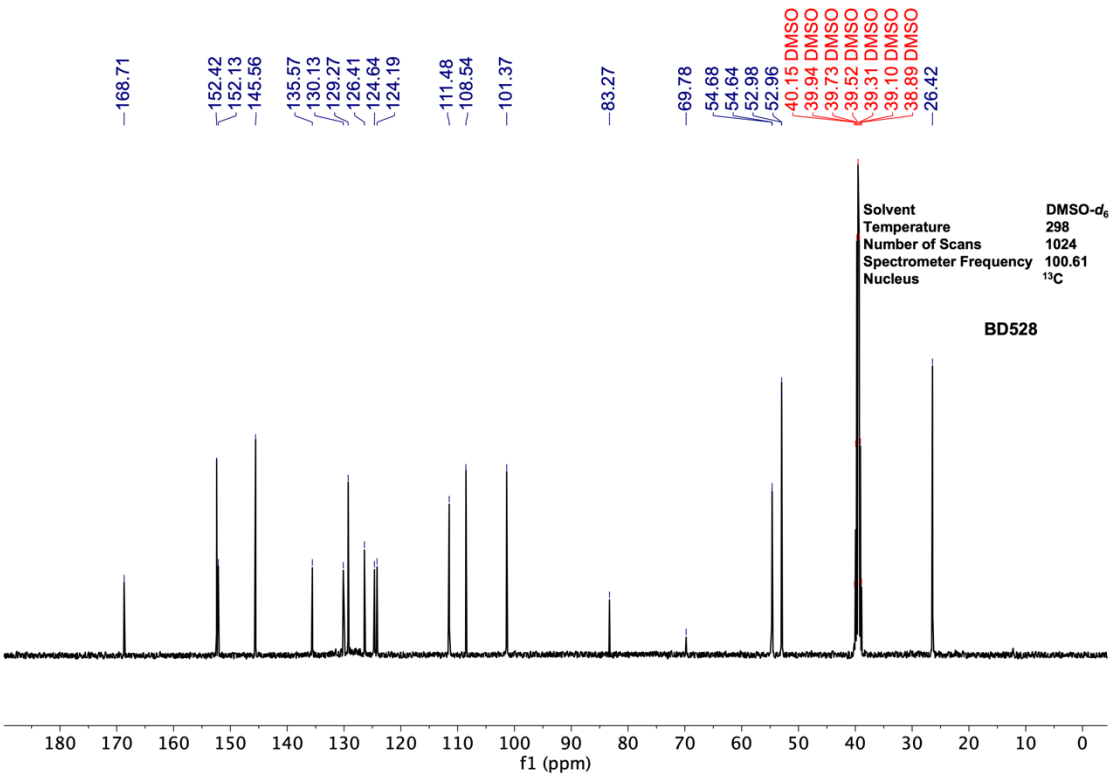
f1 (ppm)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101.37
83.27
69.78
54.68
54.64
52.98
52.96
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39.94 DMSO
39.73 DMSO
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39.31 DMSO
39.10 DMSO
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Solvent DMSO-d6
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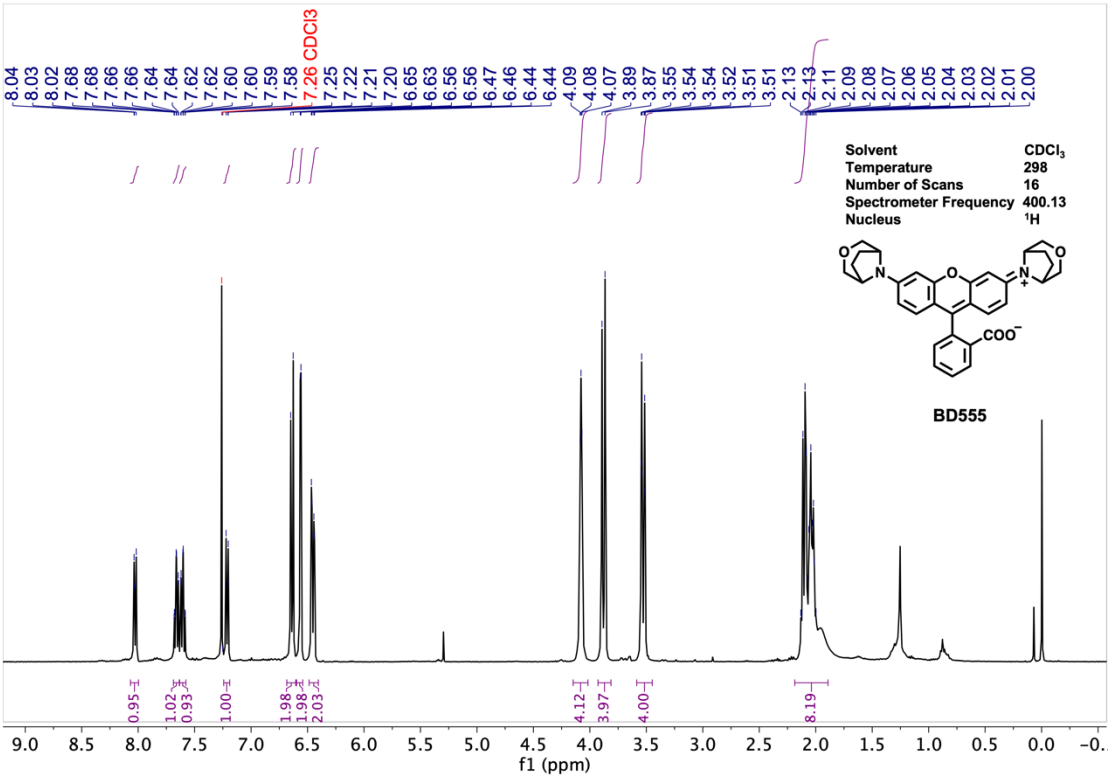
Number of Scans 1024
Spectrometer Frequency 100.61
Nucleus 13C
BD528
f1 (ppm)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Solvent CDCl3
Temperature 298
Number of Scans 16
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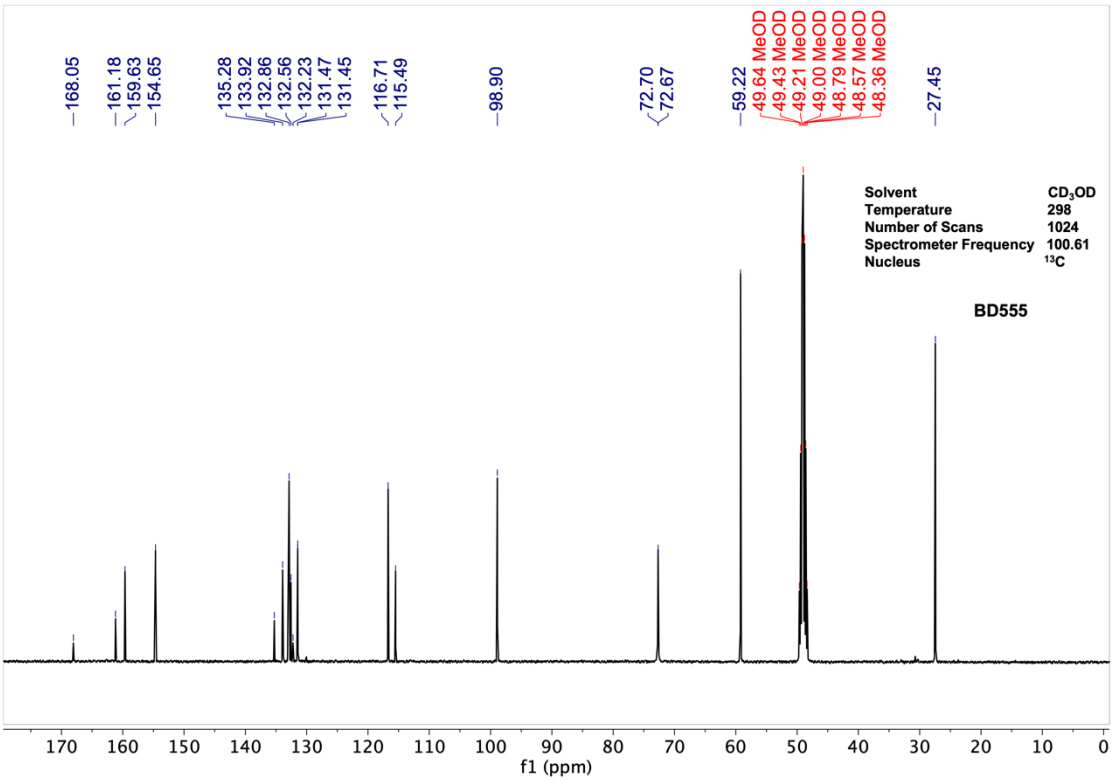
f1 (ppm)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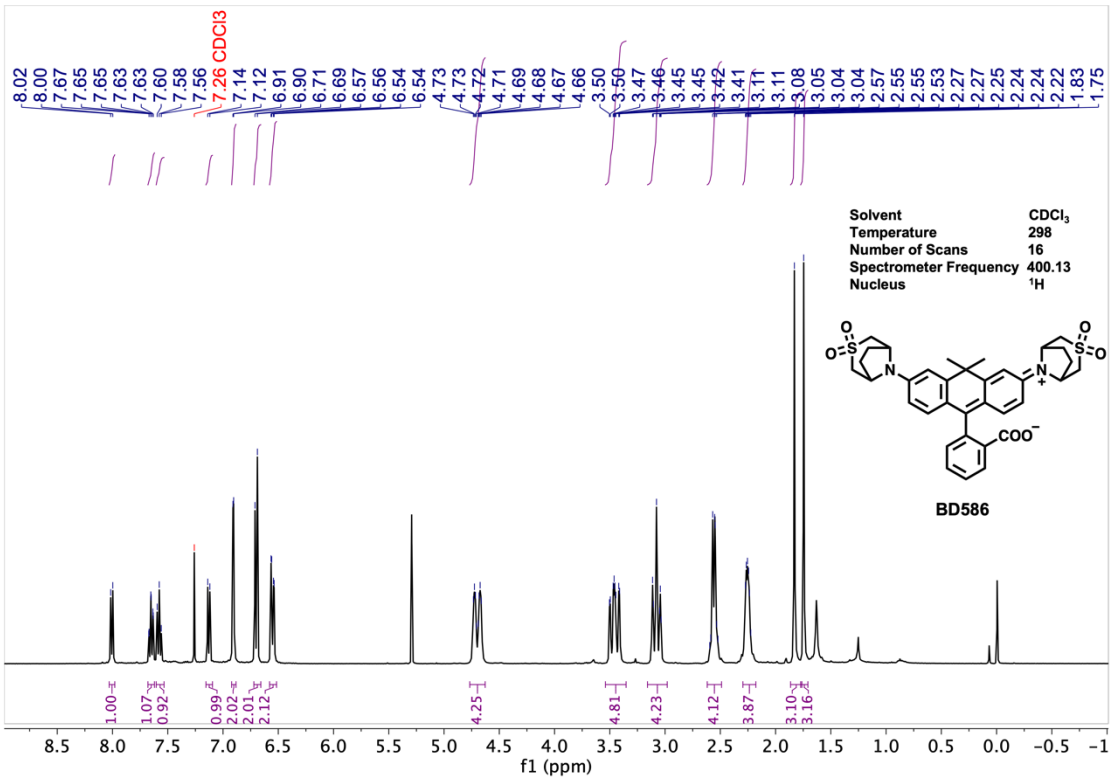
f1 (ppm)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7.56
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1.75
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
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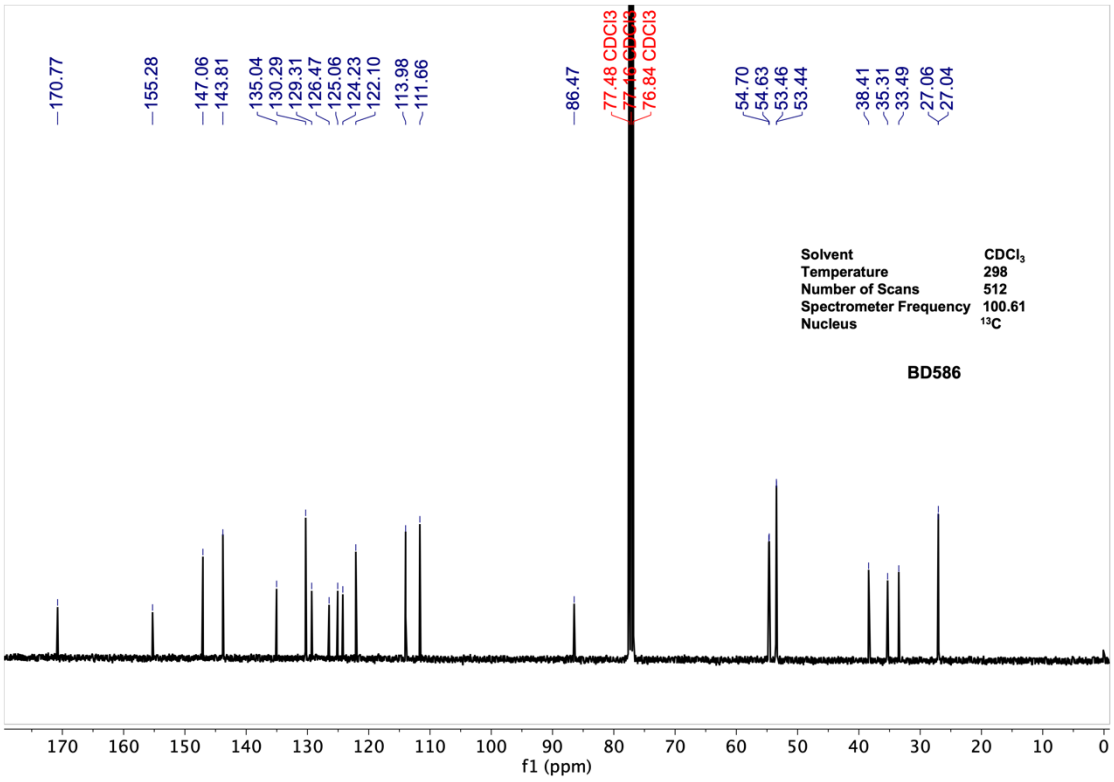
f1 (ppm)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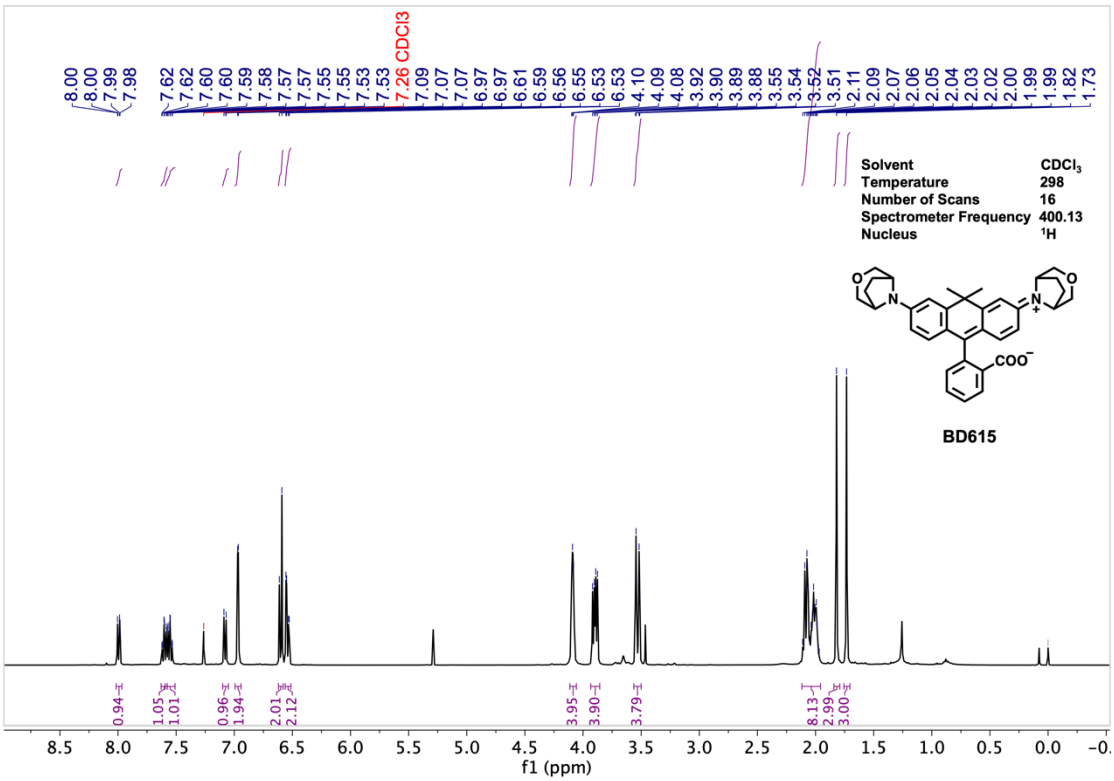
77.16 CDCl3
76.84 CDCl3
54.70
54.63
53.46
53.44
38.41
35.31
33.49
27.06
27.04
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
BD586
f1 (ppm)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7.55
7.55
7.53
7.53
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7.07
7.07
6.97
6.61
6.59
6.56
6.55
6.53
6.53
4.10
4.09
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2.04
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2.02
2.00
1.99
1.99
1.82
1.73
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
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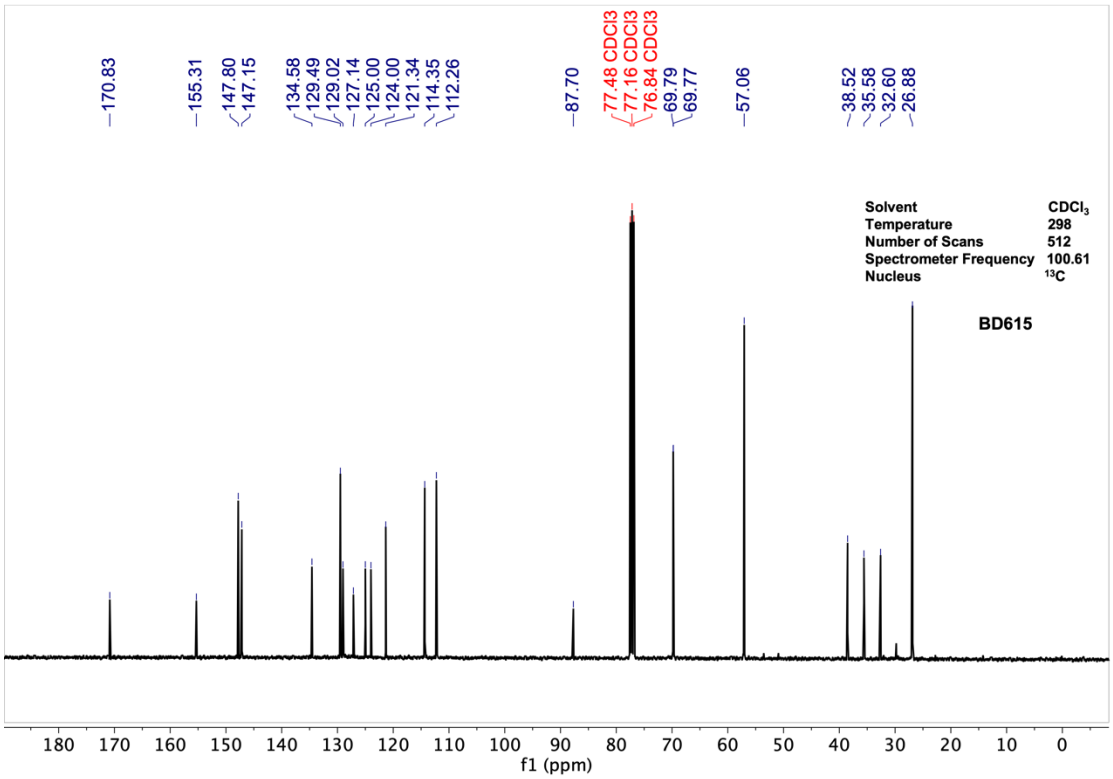
f1 (ppm)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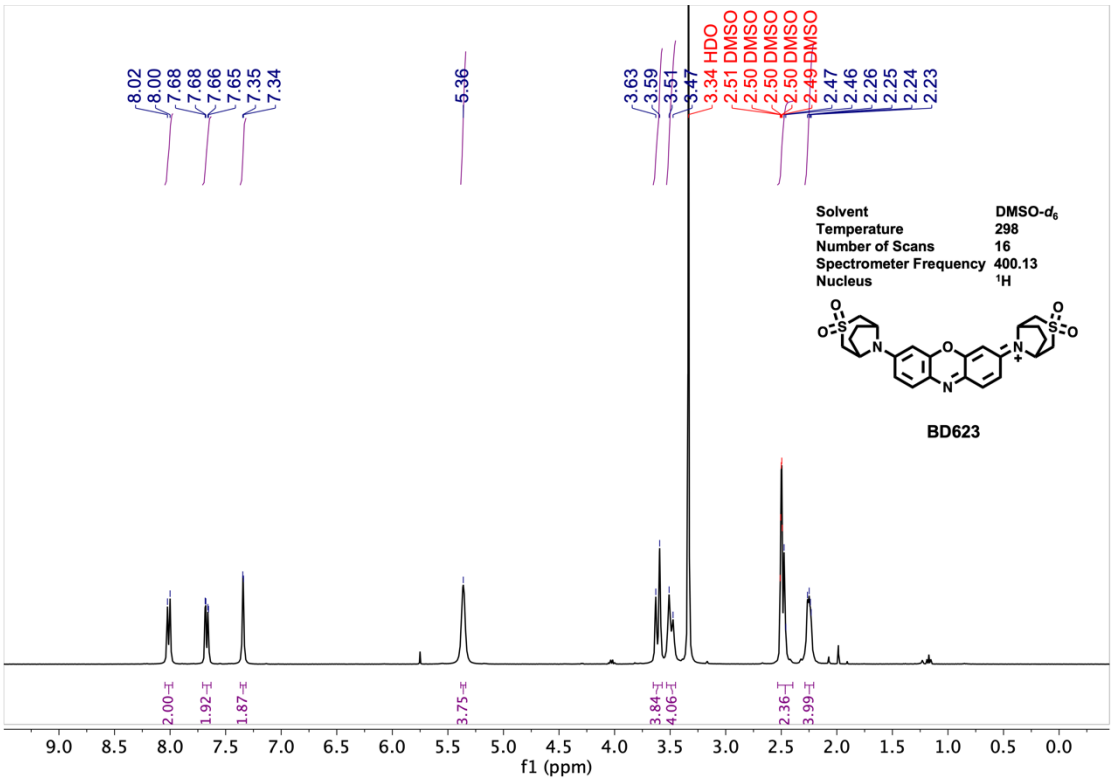
87.70
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
69.79
69.77
57.06
38.52
35.58
32.60
26.88
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
BD615
f1 (ppm)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3.59
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2.50 DMSO
2.49 DMSO
2.47
2.46
2.26
2.25
2.24
2.23
Solvent DMSO-d_6
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
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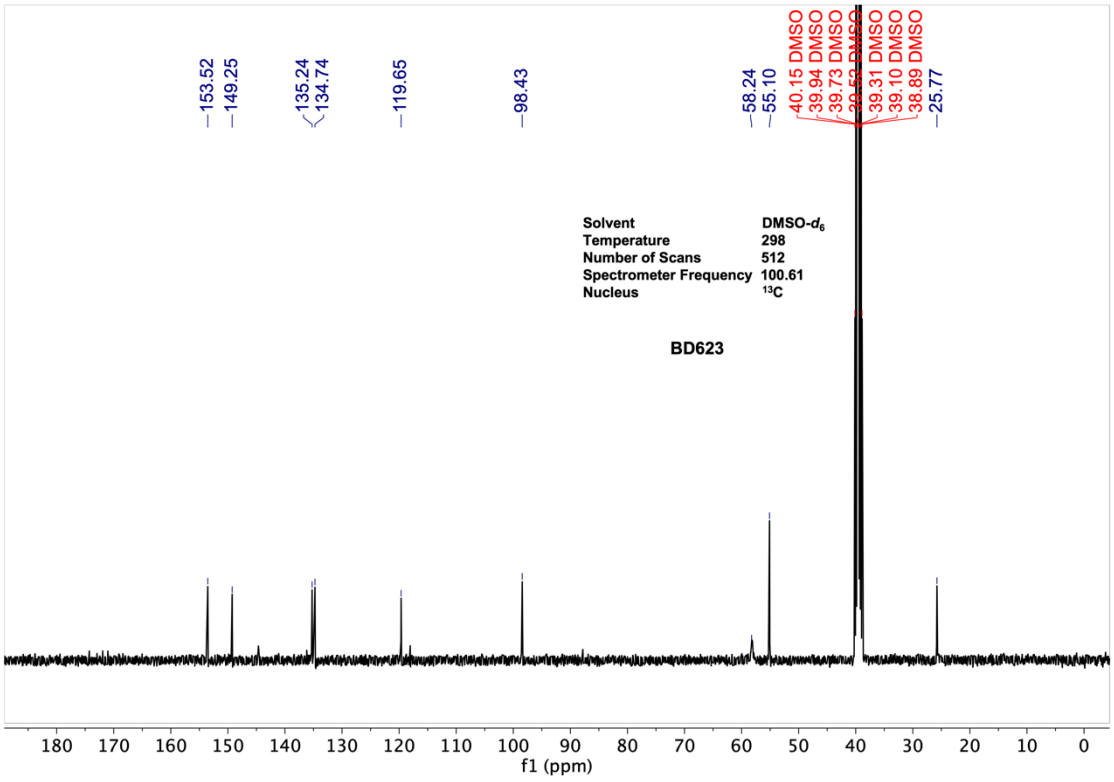
3.99
f1 (ppm)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Solvent DMSO-d_6
Temperature 298
Number of Scans 512
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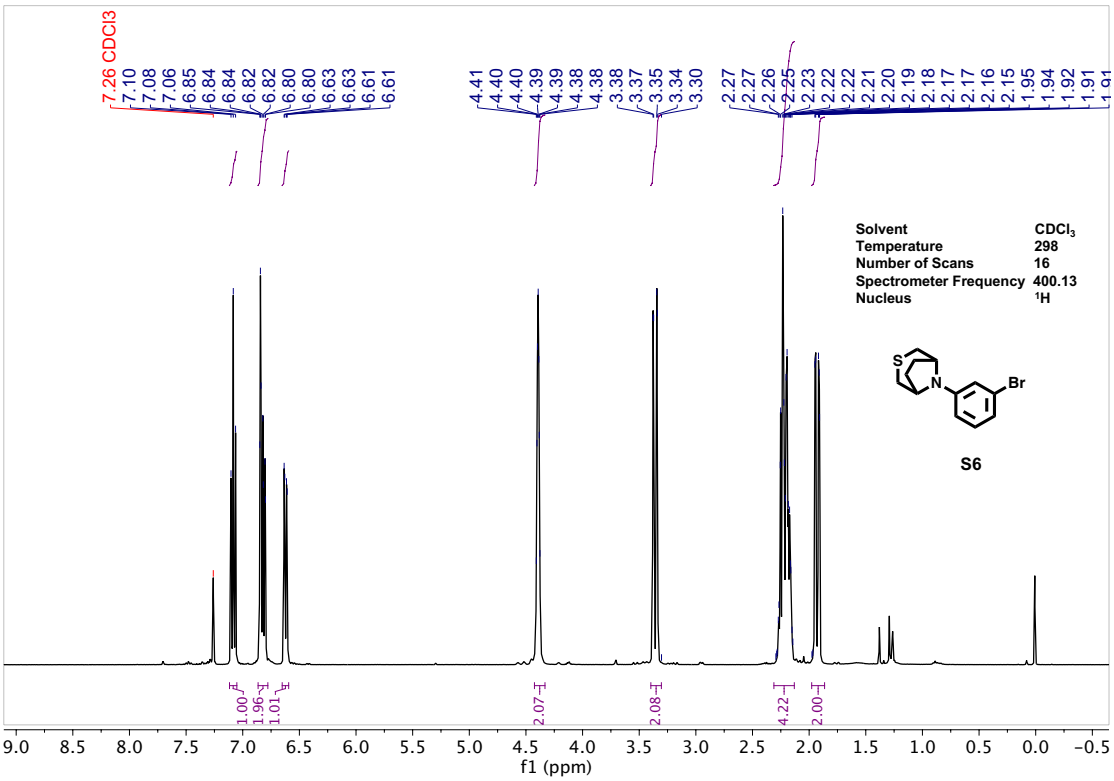
BD623
f1 (ppm)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4.39
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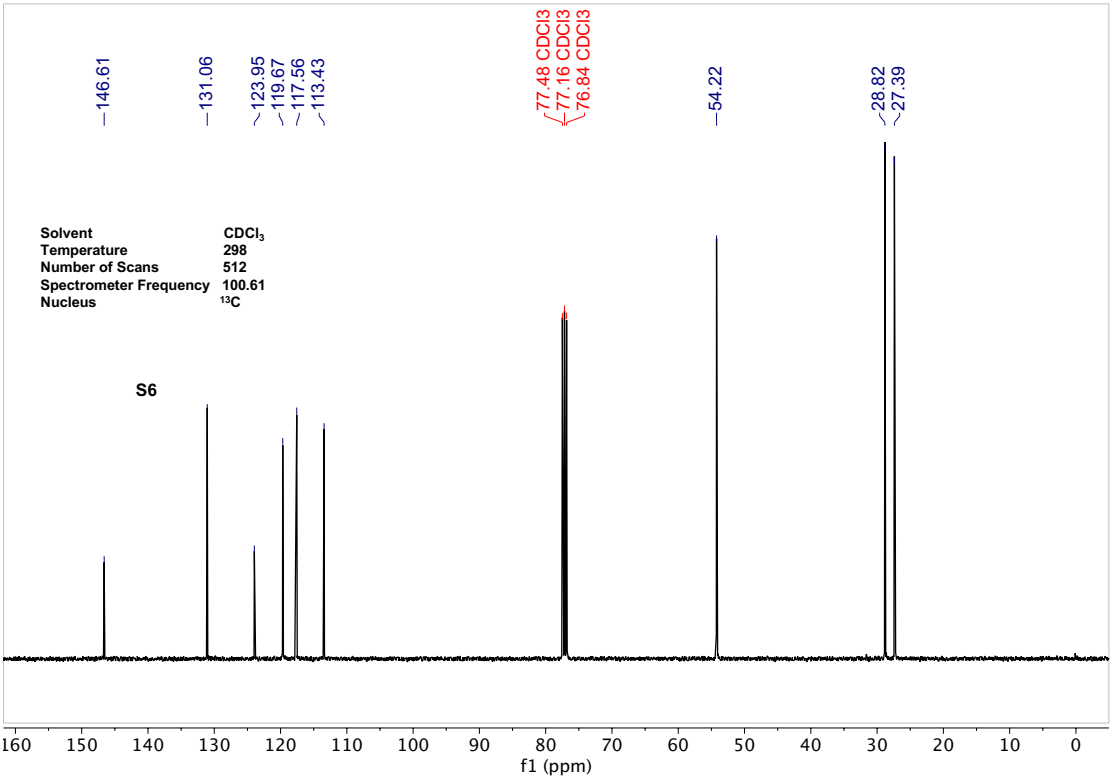
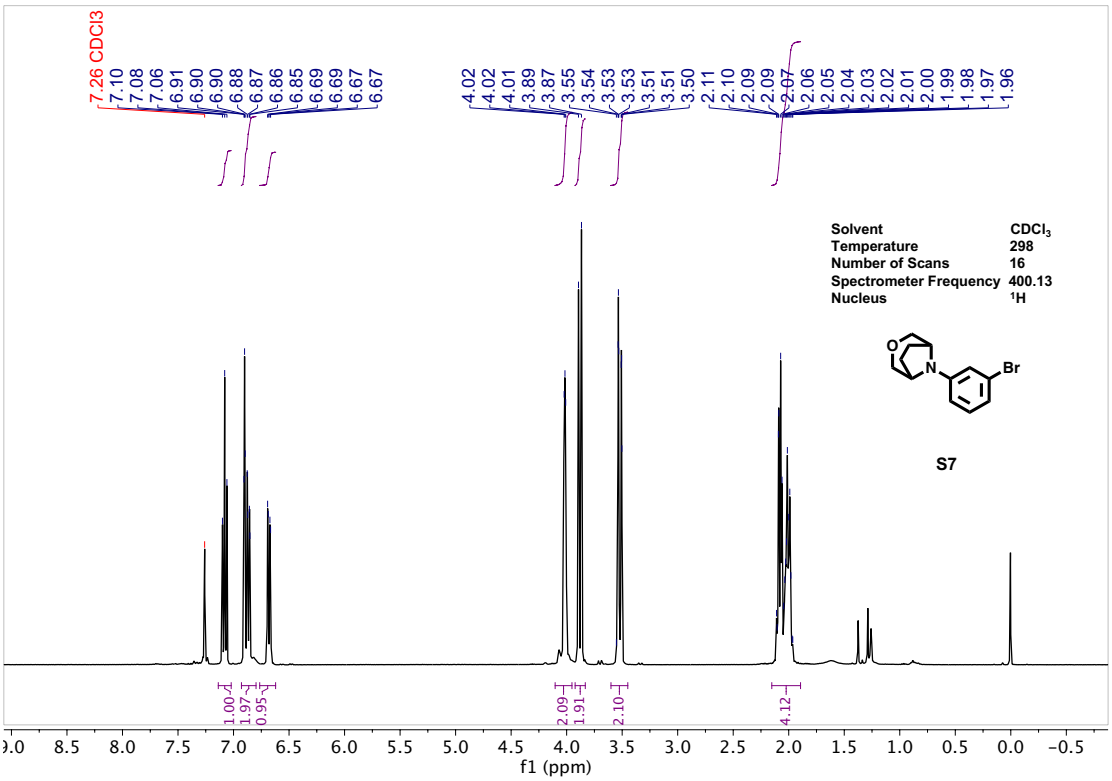
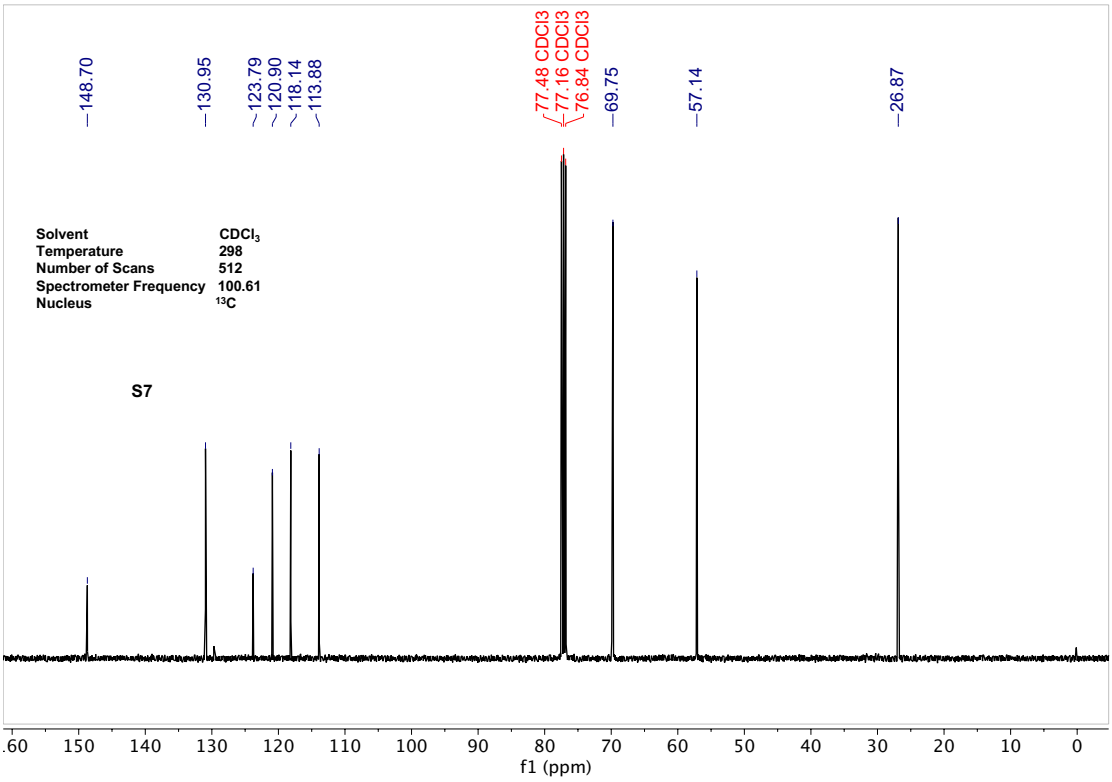
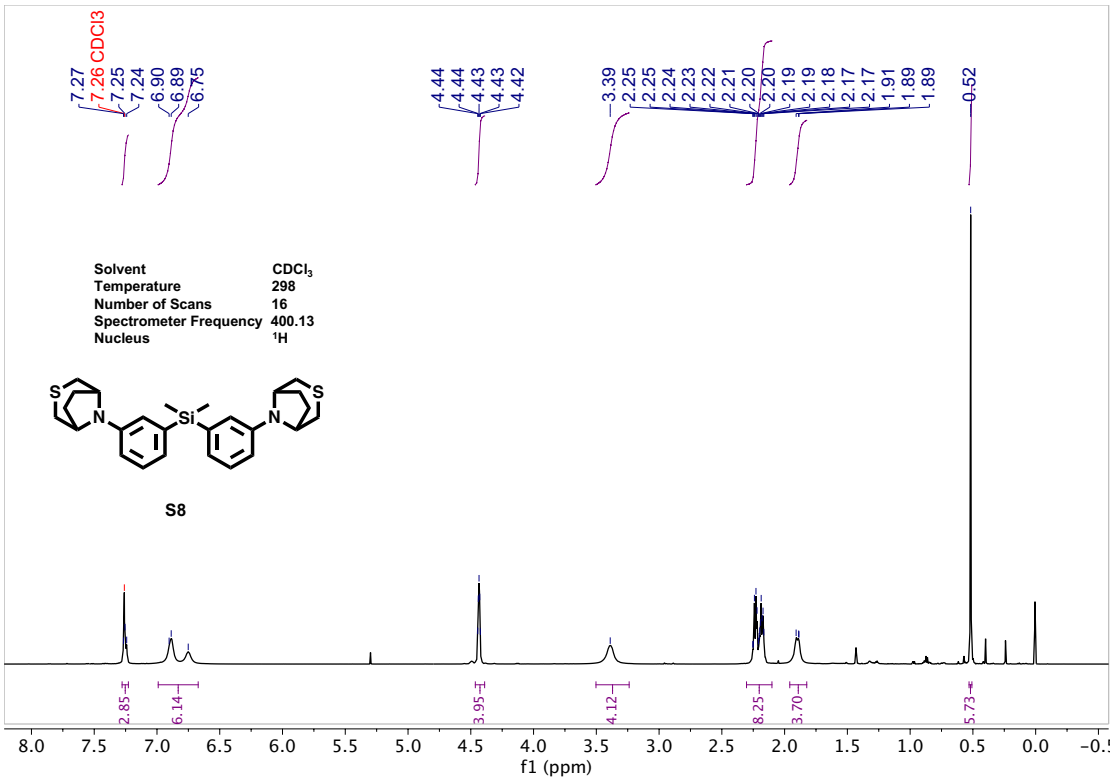
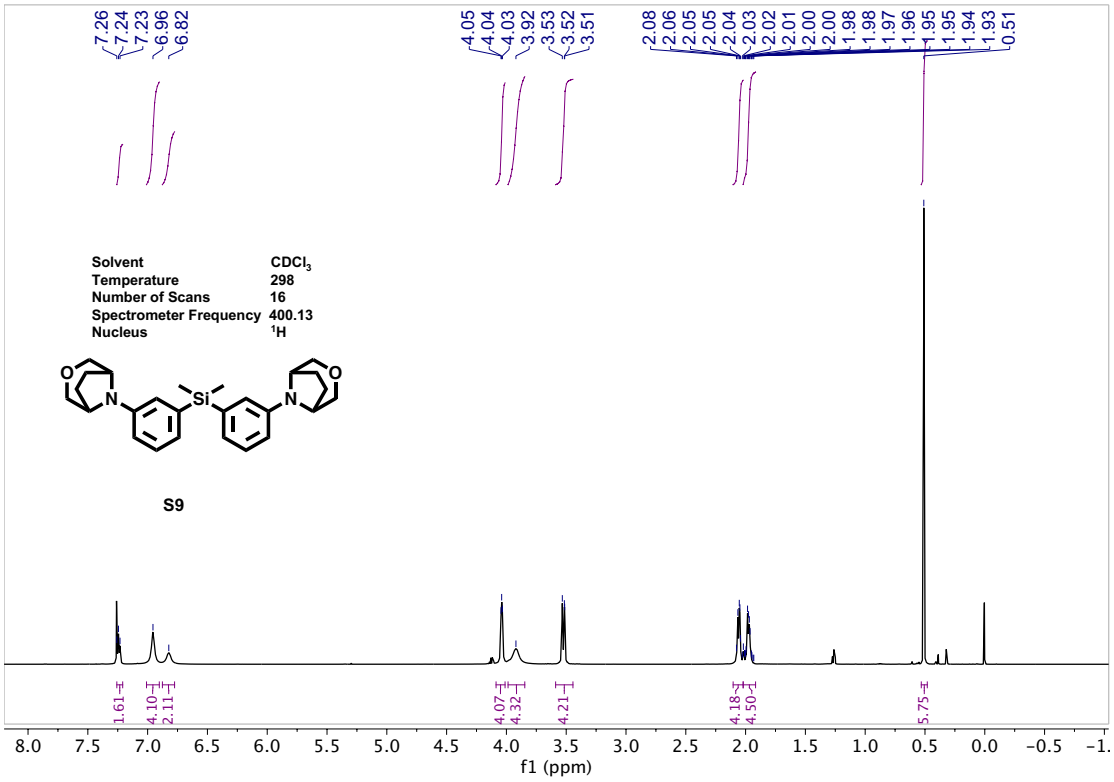
4.38
3.38
3.37
3.35
3.34
3.30
2.27
2.27
2.26
2.25
2.23
2.22
2.22
2.21
2.20
2.19
2.18
2.17
2.17
2.16
2.15
1.95
1.94
1.92
1.91
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
S
N
Br
S6
1.00
1.96
1.01
2.07
2.08
4.22
2.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 −0.5
f1 (ppm)

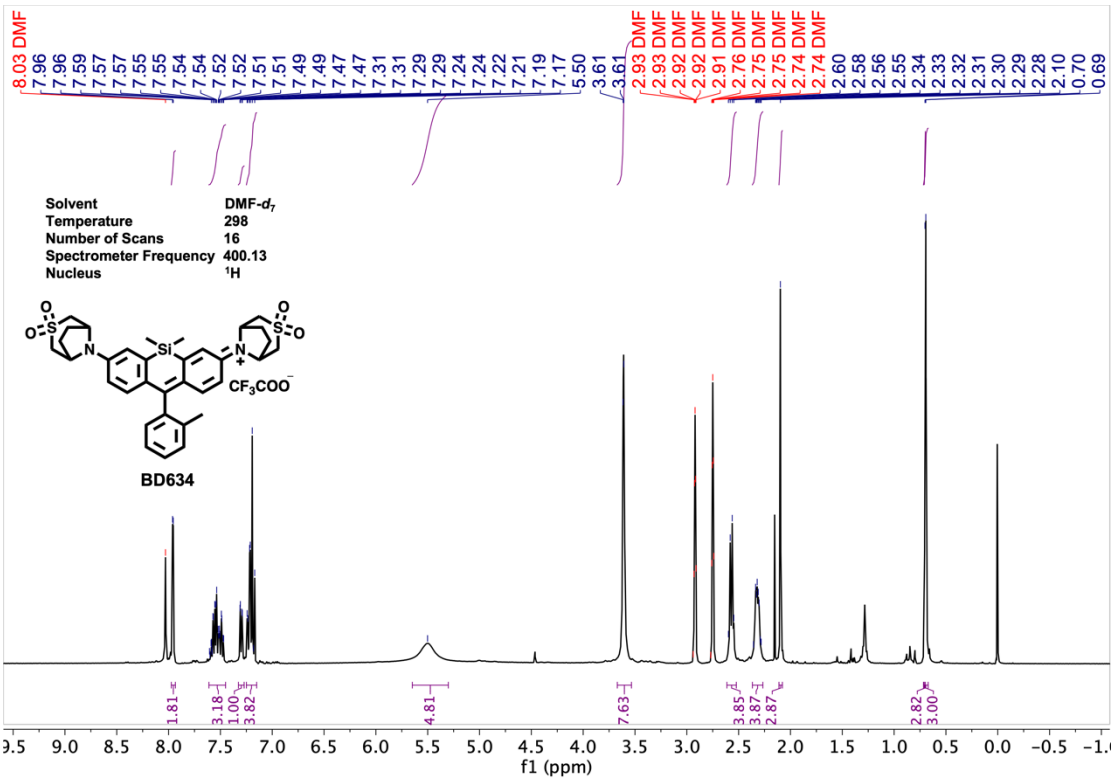

146.61
131.06
123.95
119.67
117.56
113.43
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
54.22
28.82
27.39
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
S6
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

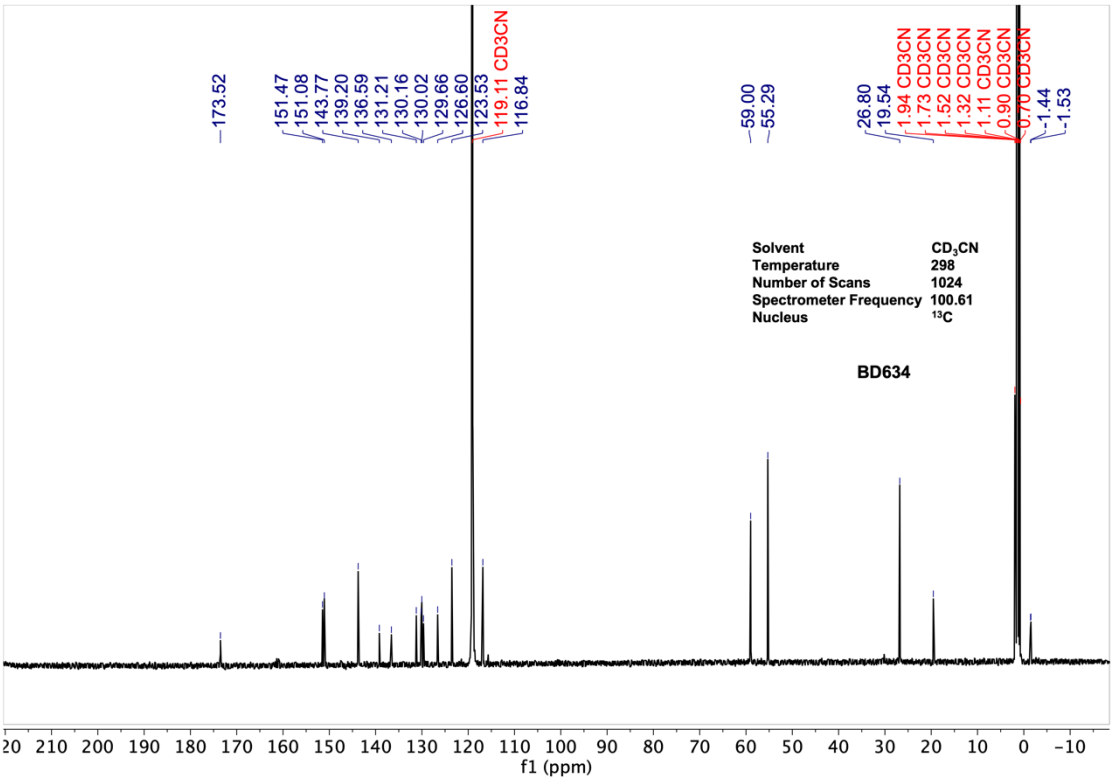

7.26 CDCl3
7.10
7.08
7.06
6.91
6.90
6.90
6.88
6.87
6.86
6.85
6.69
6.69
6.67
6.67
4.02
4.02
4.01
3.89
3.87
3.55
3.54
3.53
3.53
3.51
3.51
3.50
2.11
2.10
2.09
2.09
2.07
2.06
2.05
2.04
2.03
2.02
2.01
2.00
1.99
1.98
1.97
1.96
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
O
N
Br
S7
1.00
1.97
0.95
2.09
1.91
2.10
4.12
f1 (ppm)

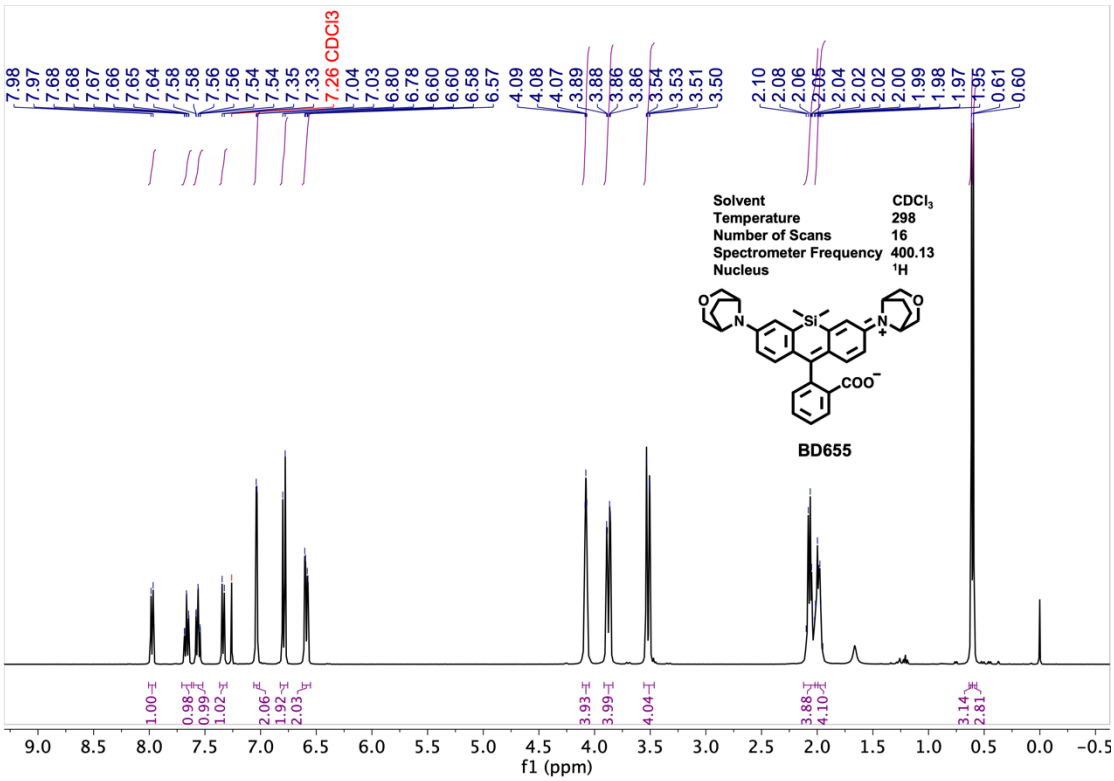

148.70
130.95
123.79
120.90
118.14
113.88
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
69.75
57.14
26.87
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
S7
f1 (ppm)

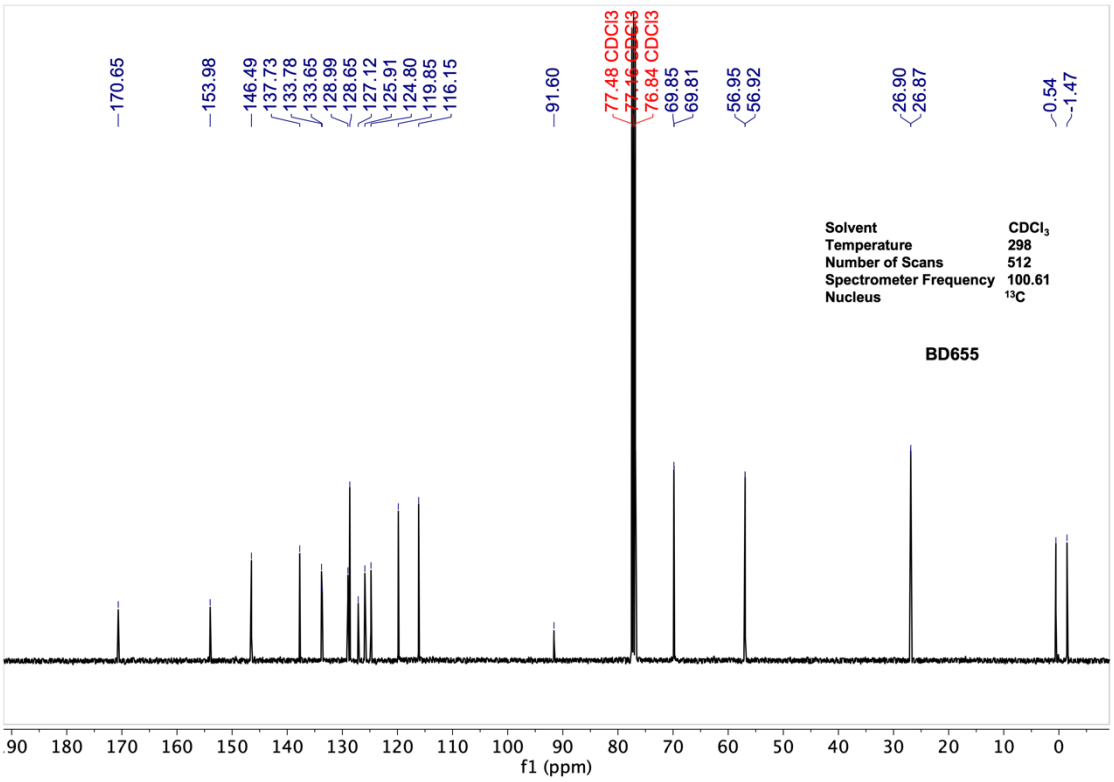

7.27
7.26 CDCl3
7.25
7.24
6.90
6.89
6.75
4.44
4.44
4.43
4.43
4.42
3.39
2.25
2.25
2.24
2.23
2.22
2.21
2.20
2.20
2.19
2.19
2.18
2.17
2.17
1.91
1.89
1.89
0.52
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
S8
2.85
6.14
3.95
4.12
8.25
3.70
5.73
f1 (ppm)

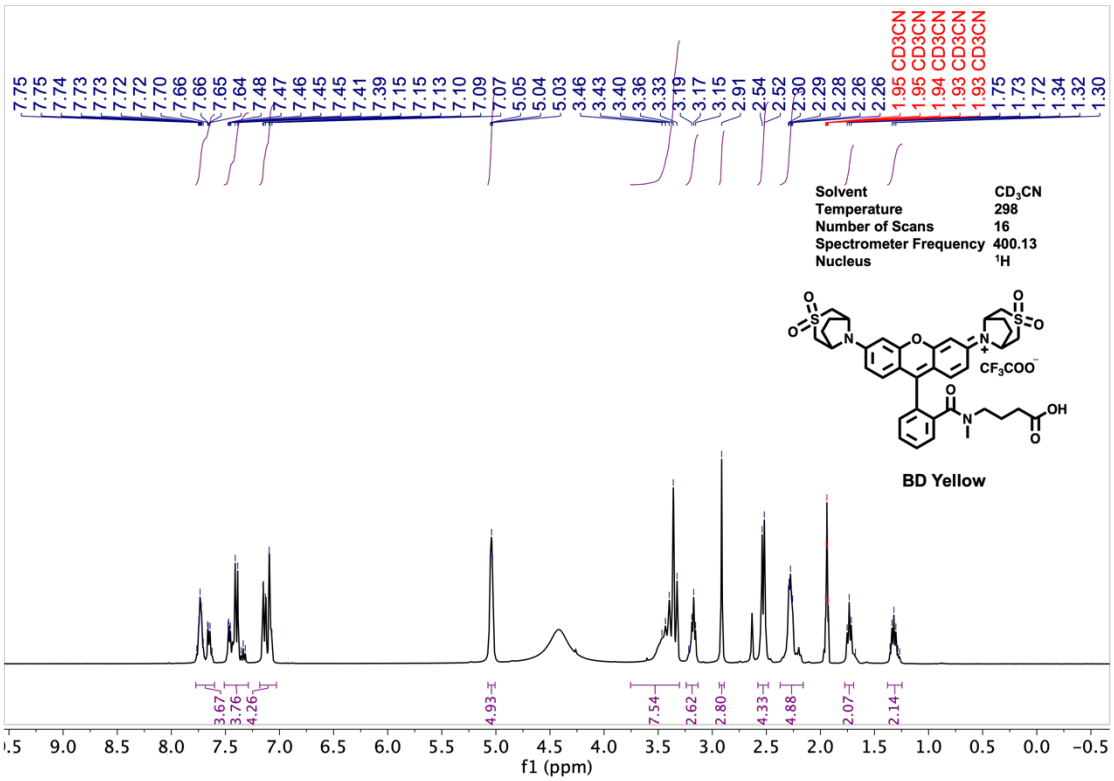

7.26
7.24
7.23
6.96
6.82
4.05
4.04
4.03
3.92
3.53
3.52
3.51
2.08
2.06
2.05
2.05
2.04
2.03
2.02
2.01
2.00
2.00
1.98
1.98
1.97
1.96
1.95
1.95
1.94
1.93
0.51
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
S9
1.61
4.10
2.11
4.07
4.32
4.21
4.18
4.50
5.75
f1 (ppm)

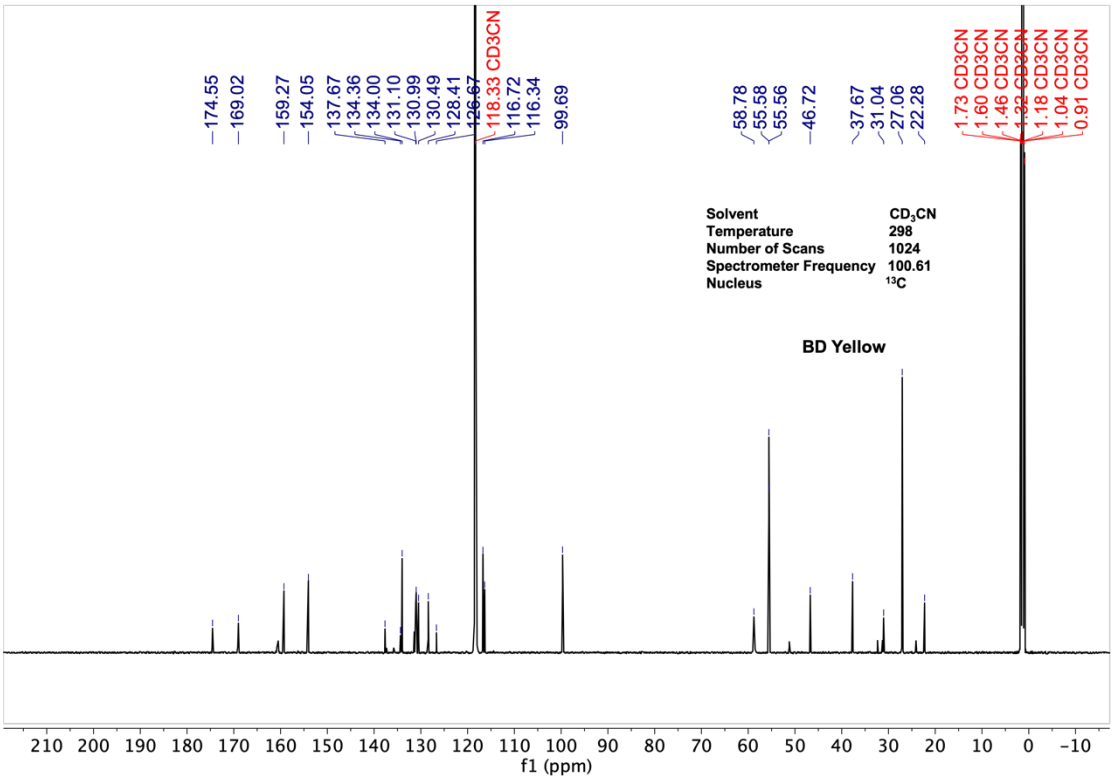

Solvent DMF-d_7
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus ^{1}H
BD634
f1 (ppm)

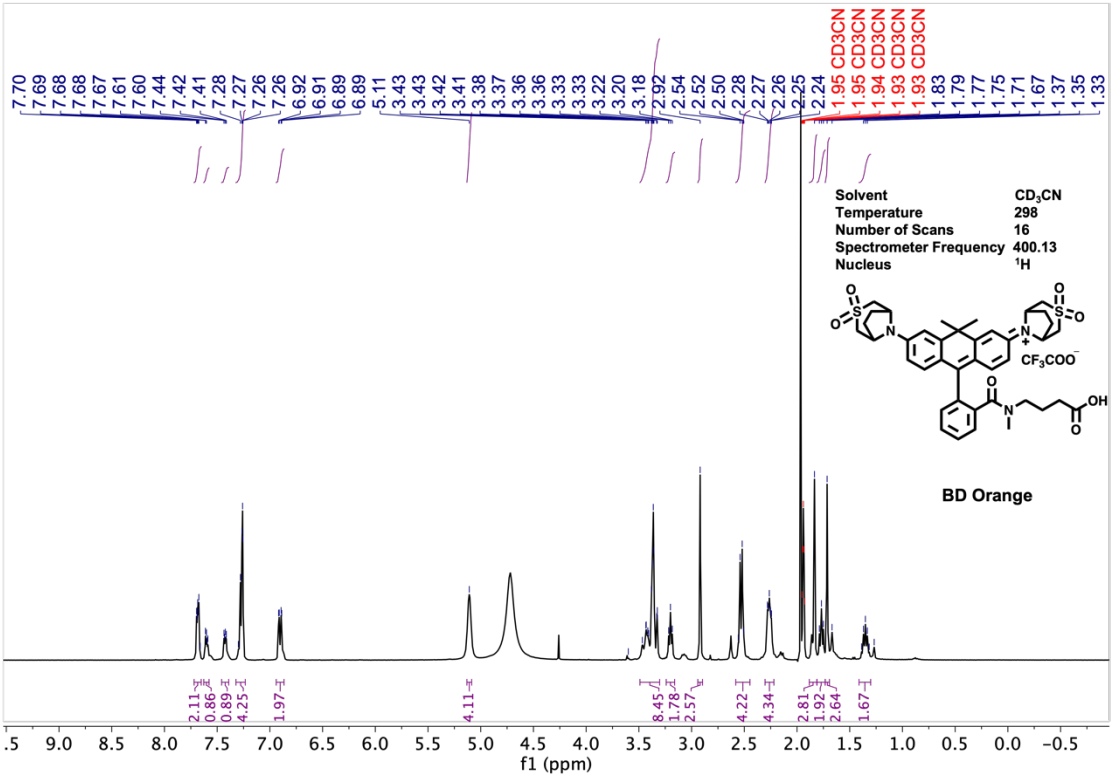

Solvent CD_3CN
Temperature 298
Number of Scans 1024
Spectrometer Frequency 100.61
Nucleus ^{13}C
BD634
f1 (ppm)

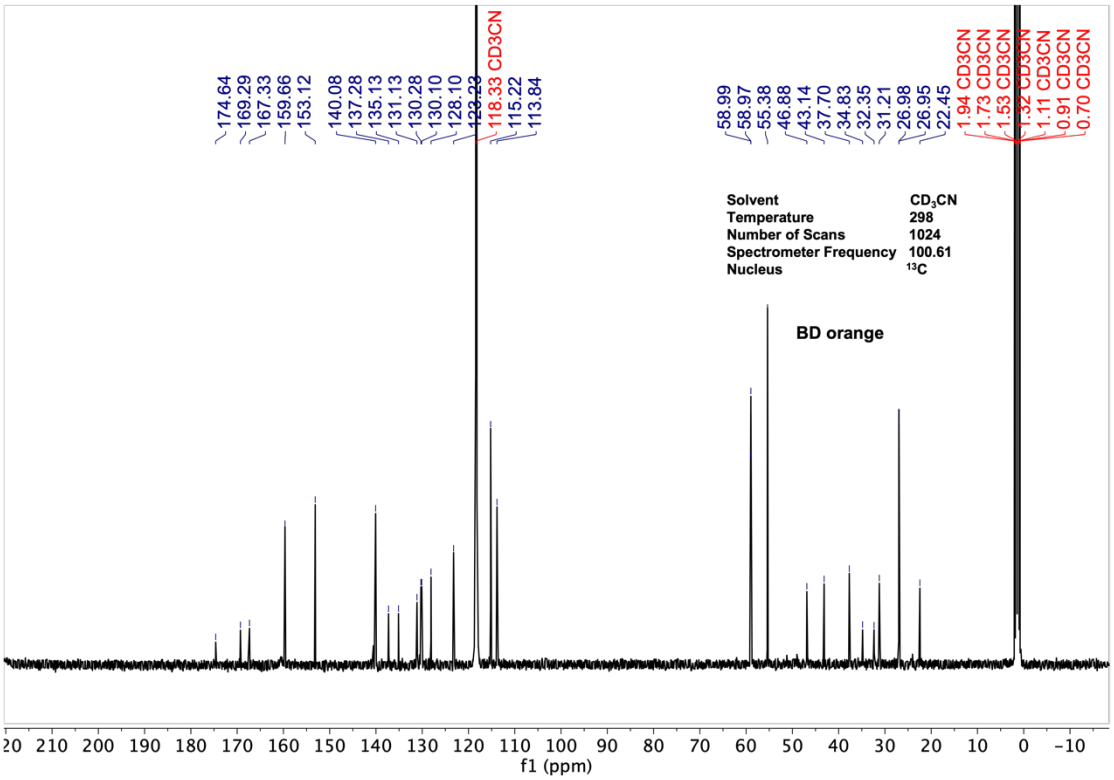

7.98 7.97 7.68 7.68 7.67 7.66 7.65 7.64 7.58 7.58 7.56 7.56 7.54 7.54 7.35 7.33 7.26 CDCl3 7.04 7.03 6.80 6.78 6.60 6.60 6.58 6.57
4.09 4.08 4.07 3.89 3.88 3.86 3.86 3.54 3.53 3.51 3.50
2.10 2.08 2.06 2.05 2.04 2.02 2.02 2.00 1.99 1.98 1.97 1.95 0.61 0.60
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
BD655
1.00 0.98 0.99 1.02 2.06 1.92 2.03 3.93 3.99 4.04 3.88 4.10 3.14 2.81
f1 (ppm)

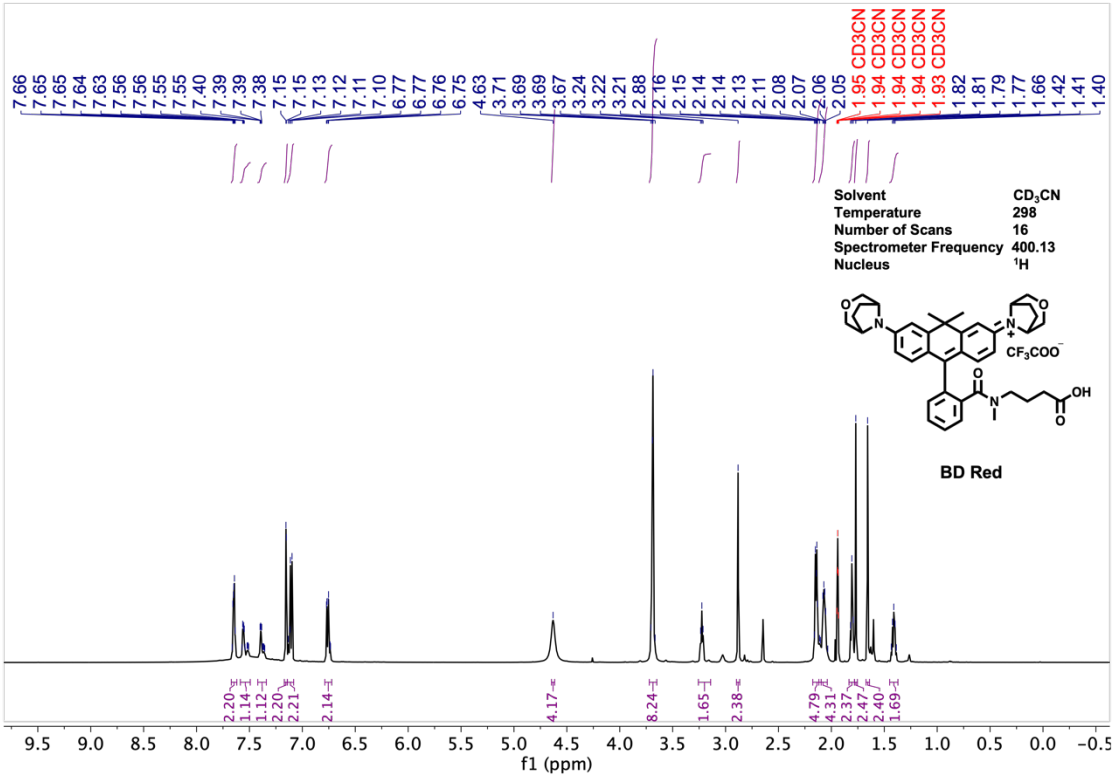

170.65 153.98 146.49 137.73 133.78 133.65 128.99 128.65 127.12 125.91 124.80 119.85 116.15 91.60 77.48 CDCl3 77.16 CDCl3 76.84 CDCl3 69.85 69.81 56.95 56.92 26.90 26.87 0.54 -1.47
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
BD655
f1 (ppm)

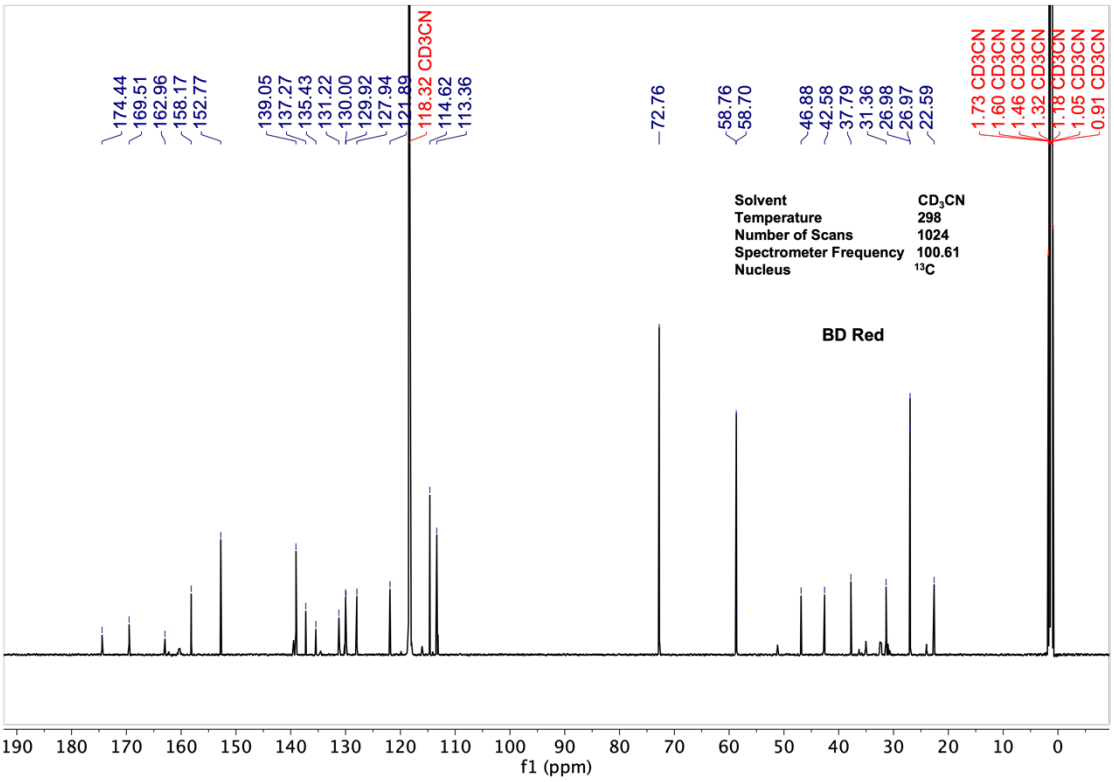

Solvent CD3CN
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
BD Yellow
f1 (ppm)

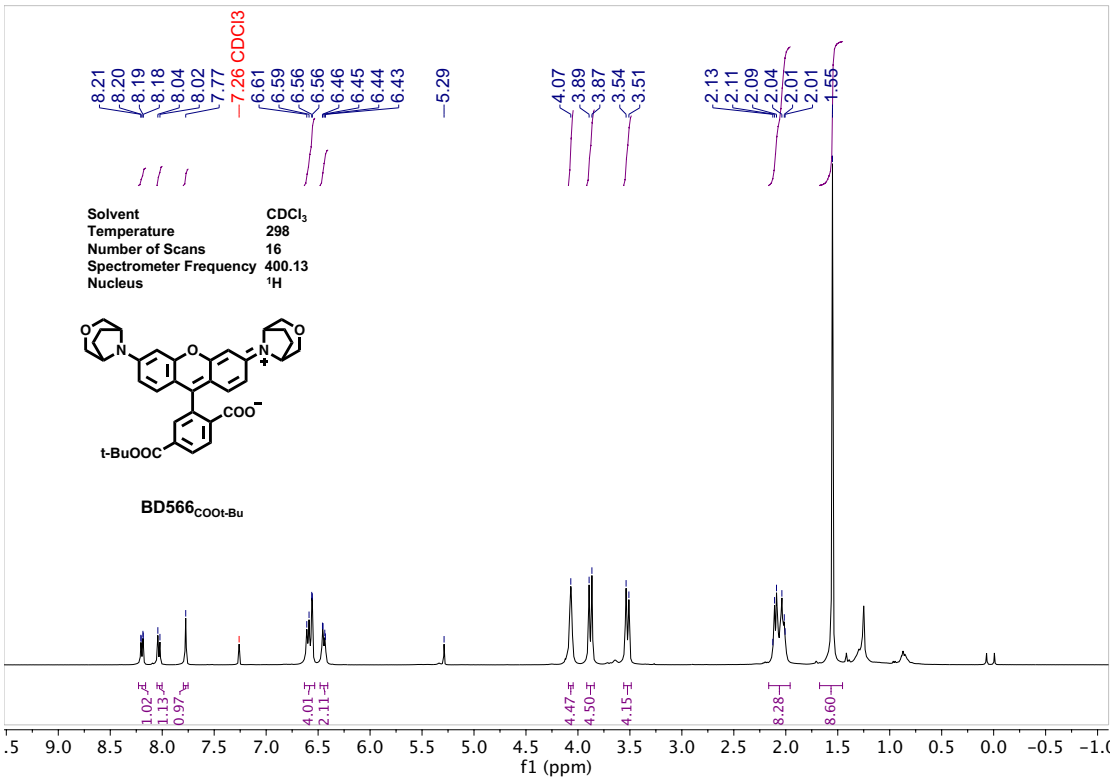

Solvent CD3CN
Temperature 298
Number of Scans 1024
Spectrometer Frequency 100.61
Nucleus 13C
BD Yellow
f1 (ppm)

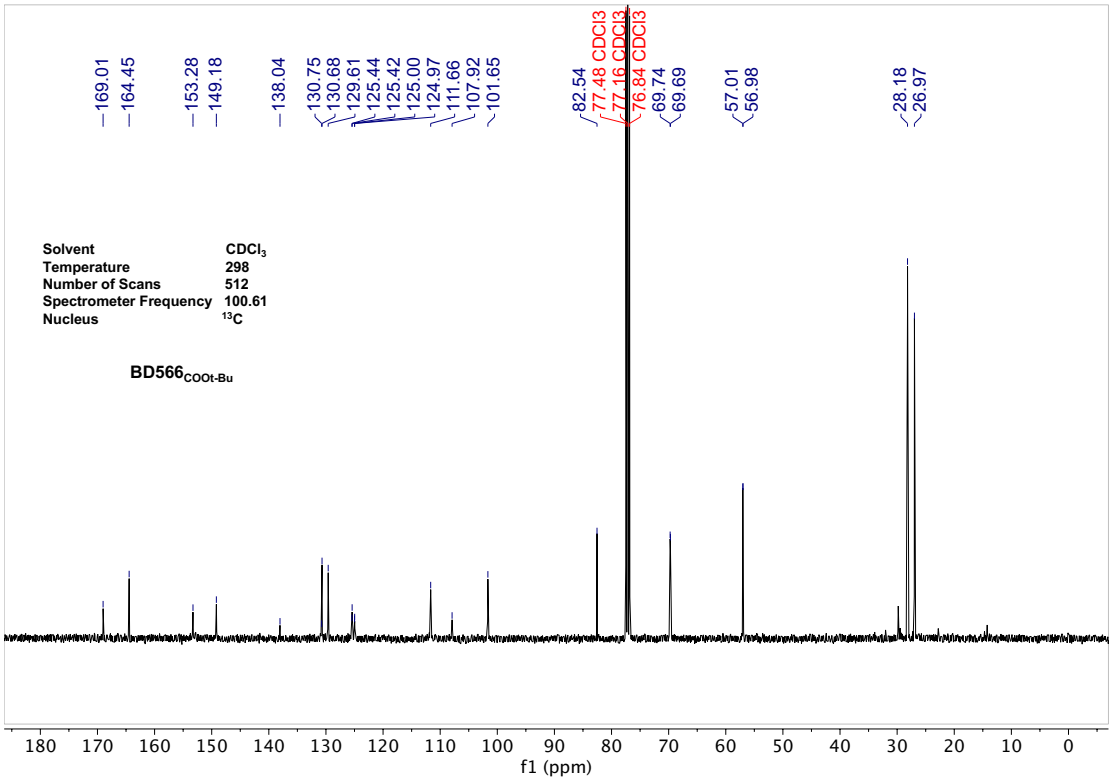

Solvent CD3CN
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
BD Orange
f1 (ppm)

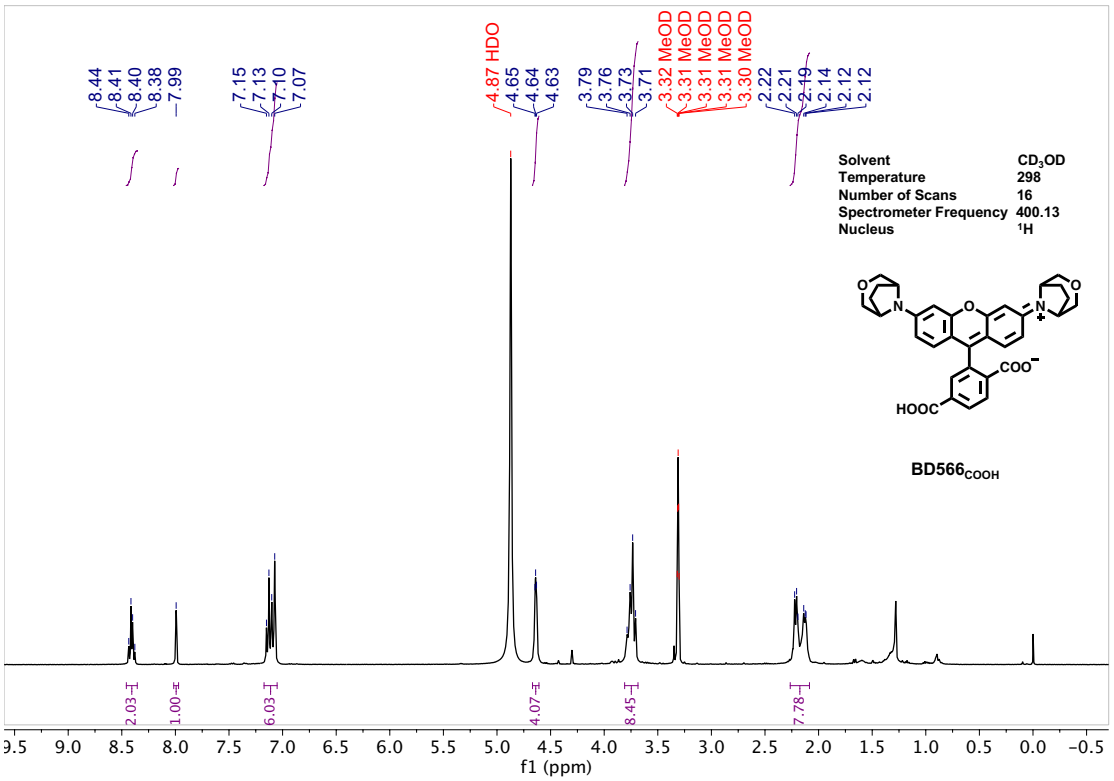

Solvent CD3CN
Temperature 298
Number of Scans 1024
Spectrometer Frequency 100.61
Nucleus 13C
BD orange
f1 (ppm)

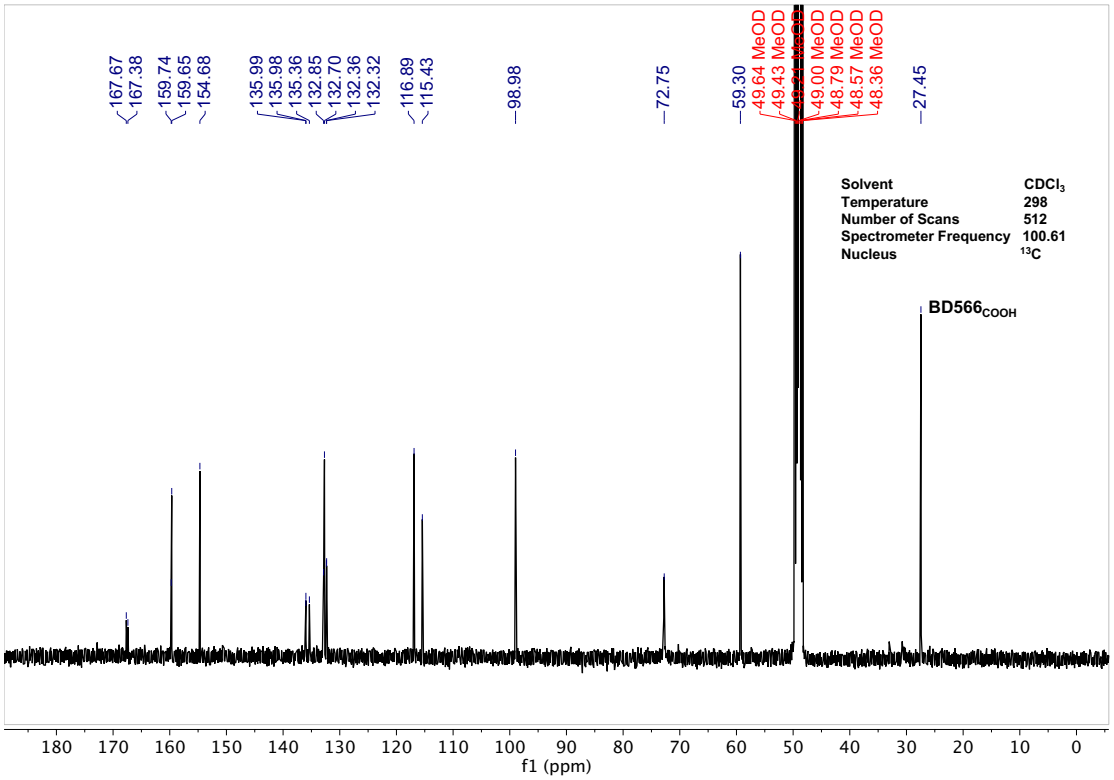

Solvent CD3CN
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
BD Red
f1 (ppm)

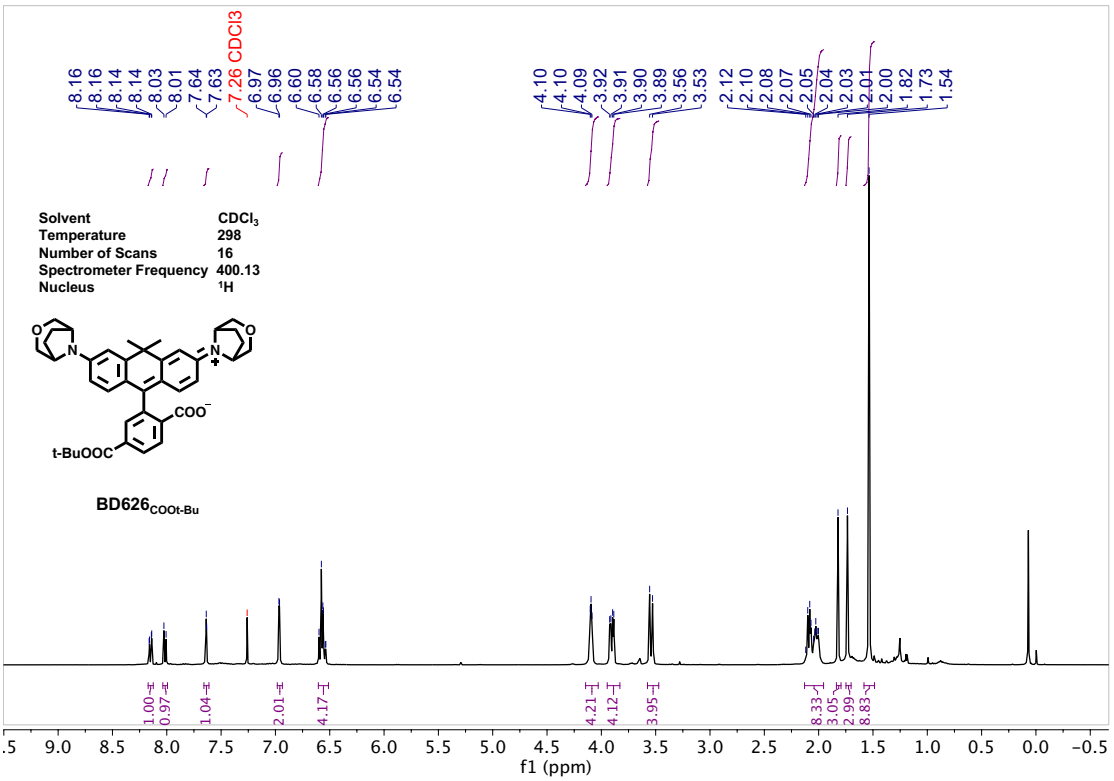

Solvent CD3CN
Temperature 298
Number of Scans 1024
Spectrometer Frequency 100.61
Nucleus 13C
BD Red
f1 (ppm)

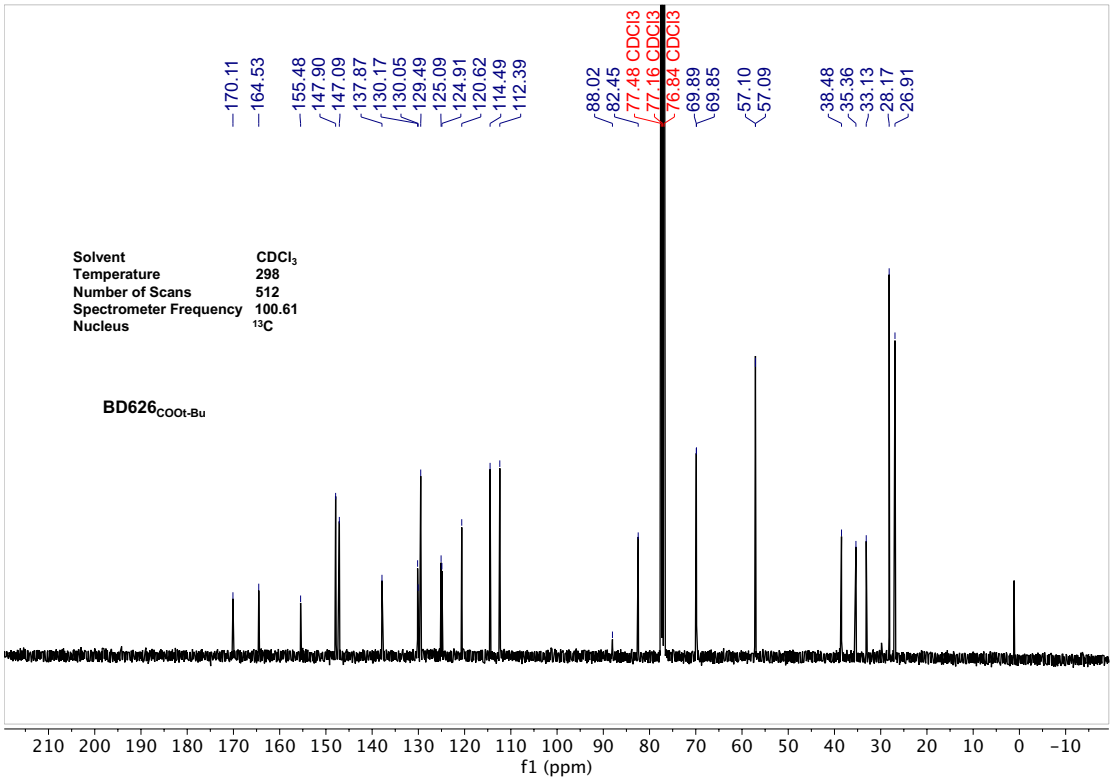

8.21
8.20
8.19
8.18
8.04
8.02
7.77
7.26 CDCl3
6.61
6.59
6.56
6.56
6.46
6.45
6.44
6.43
5.29
4.07
3.89
3.87
3.54
3.51
2.13
2.11
2.09
2.04
2.01
2.01
1.55
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
t-BuOOC
BD566COOt-Bu
1.02
1.13
0.97
4.01
2.11
4.47
4.50
4.15
8.28
8.60
f1 (ppm)

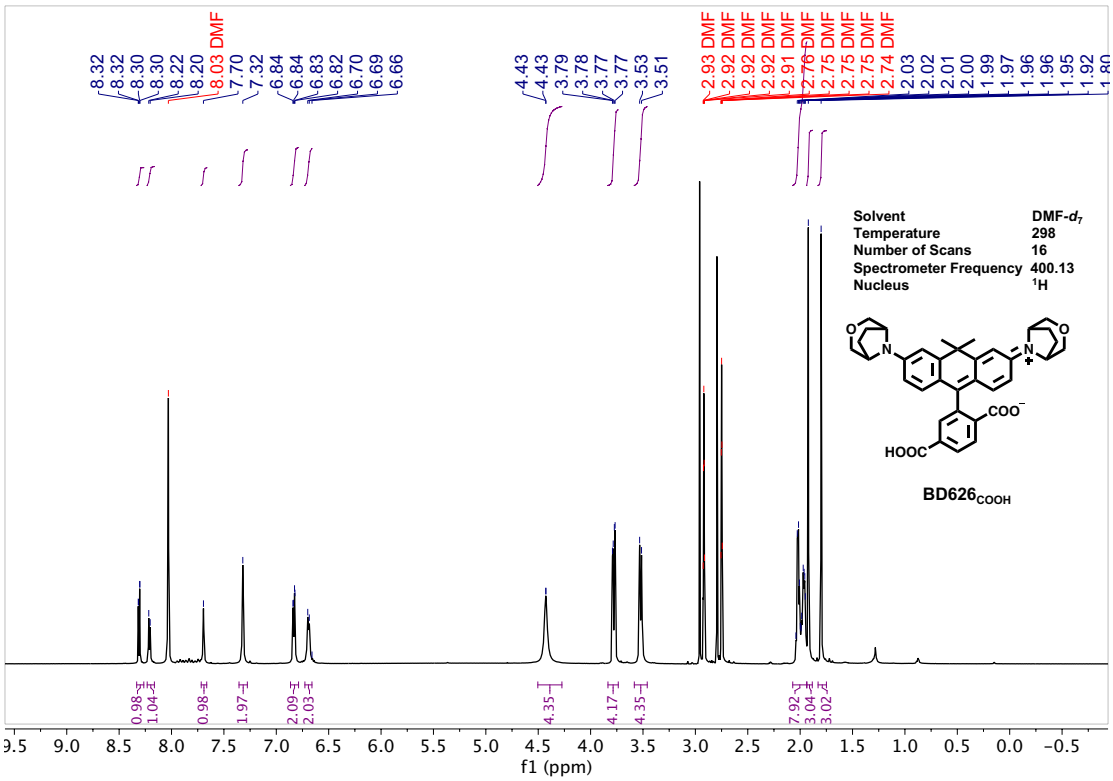

169.01
164.45
153.28
149.18
138.04
130.75
130.68
129.61
125.44
125.42
125.00
124.97
111.66
107.92
101.65
82.54
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
69.74
69.69
57.01
56.98
28.18
26.97
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
BD566COOt-Bu
f1 (ppm)

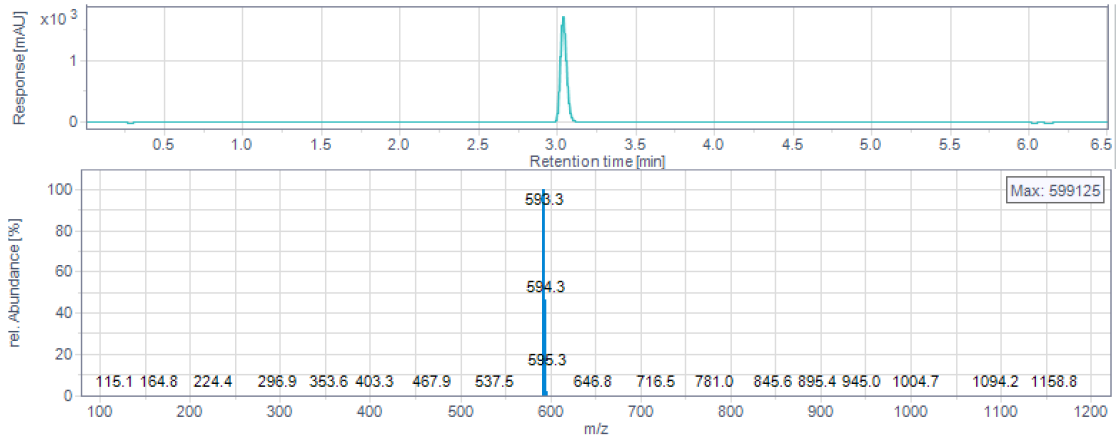

8.44
8.41
8.40
8.38
7.99
7.15
7.13
7.10
7.07
4.87 HDO
4.65
4.64
4.63
3.79
3.76
3.73
3.71
3.32 MeOD
3.31 MeOD
3.31 MeOD
3.31 MeOD
3.30 MeOD
2.22
2.21
2.19
2.14
2.12
2.12
Solvent CD3OD
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
BD566COOH
2.03
1.00
6.03
4.07
8.45
7.78
f1 (ppm)

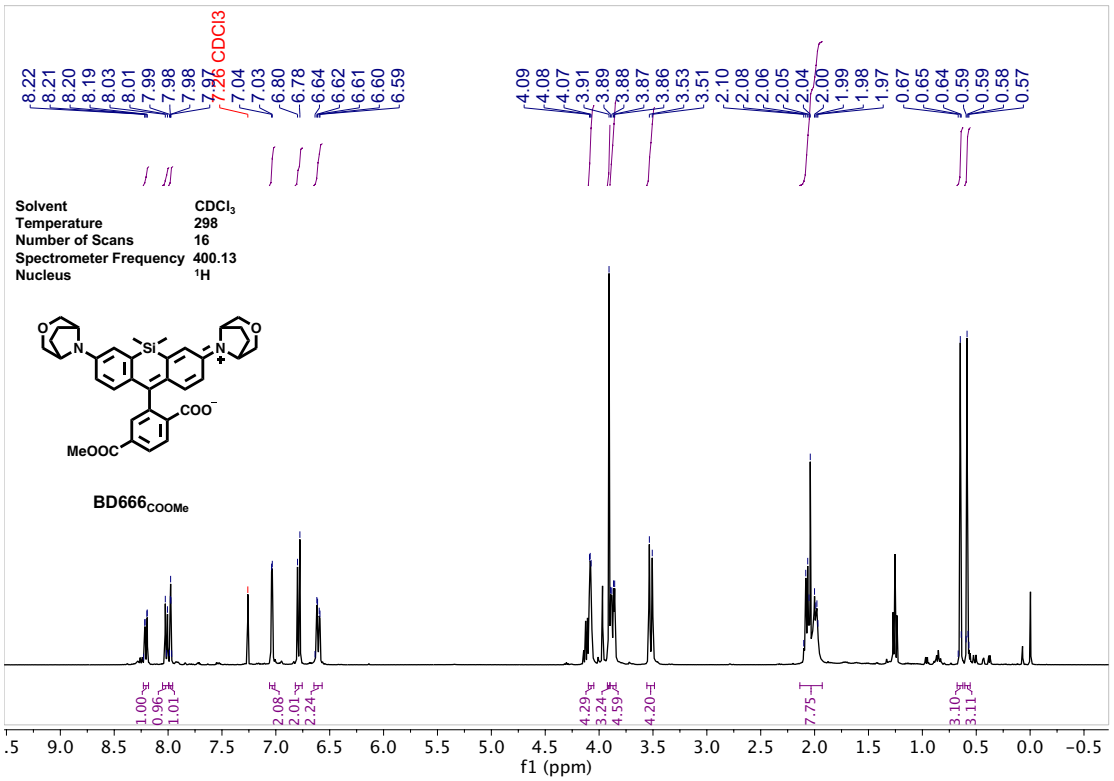

167.67
167.38
159.74
159.65
154.68
135.99
135.98
135.36
132.85
132.70
132.36
132.32
116.89
115.43
98.98
72.75
59.30
49.64 MeOD
49.43 MeOD
49.21 MeOD
49.00 MeOD
48.79 MeOD
48.57 MeOD
48.36 MeOD
27.45
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
BD566COOH
f1 (ppm)

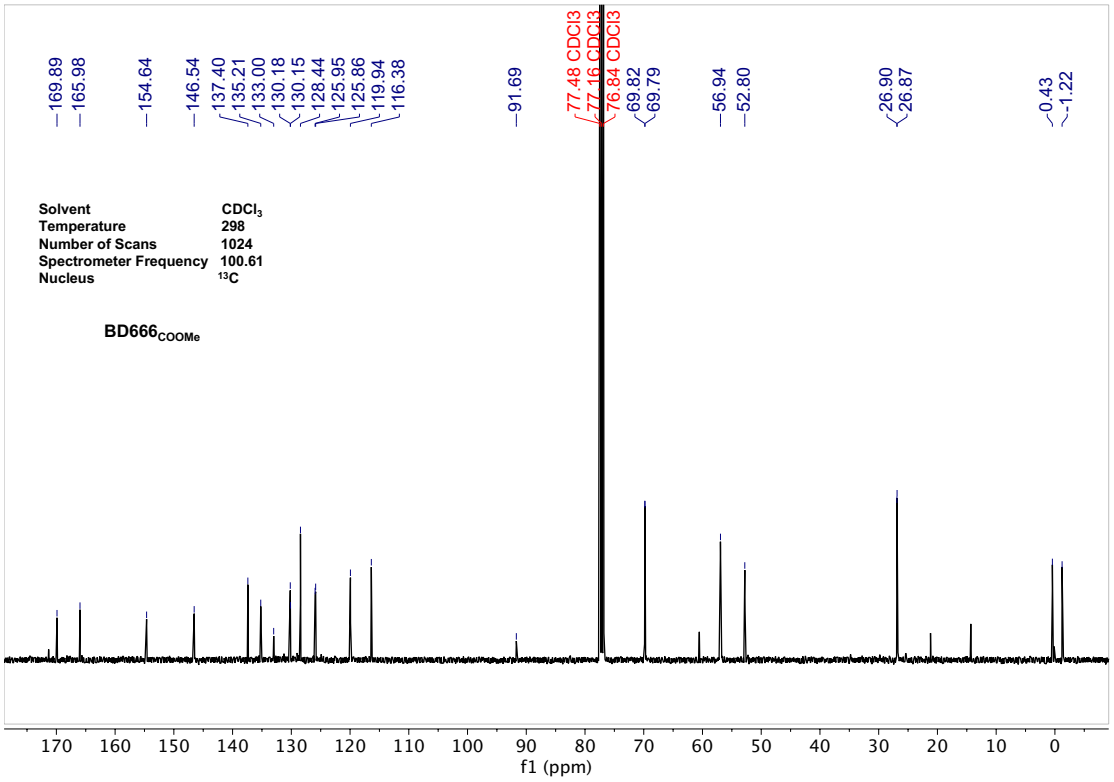

8.16
8.16
8.14
8.14
8.03
8.01
7.64
7.63
7.26 CDCl3
6.97
6.96
6.60
6.58
6.56
6.56
6.54
6.54
4.10
4.10
4.09
3.92
3.91
3.90
3.89
3.56
3.53
2.12
2.10
2.08
2.07
2.05
2.04
2.03
2.01
2.00
1.82
1.73
1.54
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
COO−
t-BuOOC
BD626COOt-Bu
1.00
0.97
1.04
2.01
4.17
4.21
4.12
3.95
8.33
3.05
2.99
8.83
f1 (ppm)

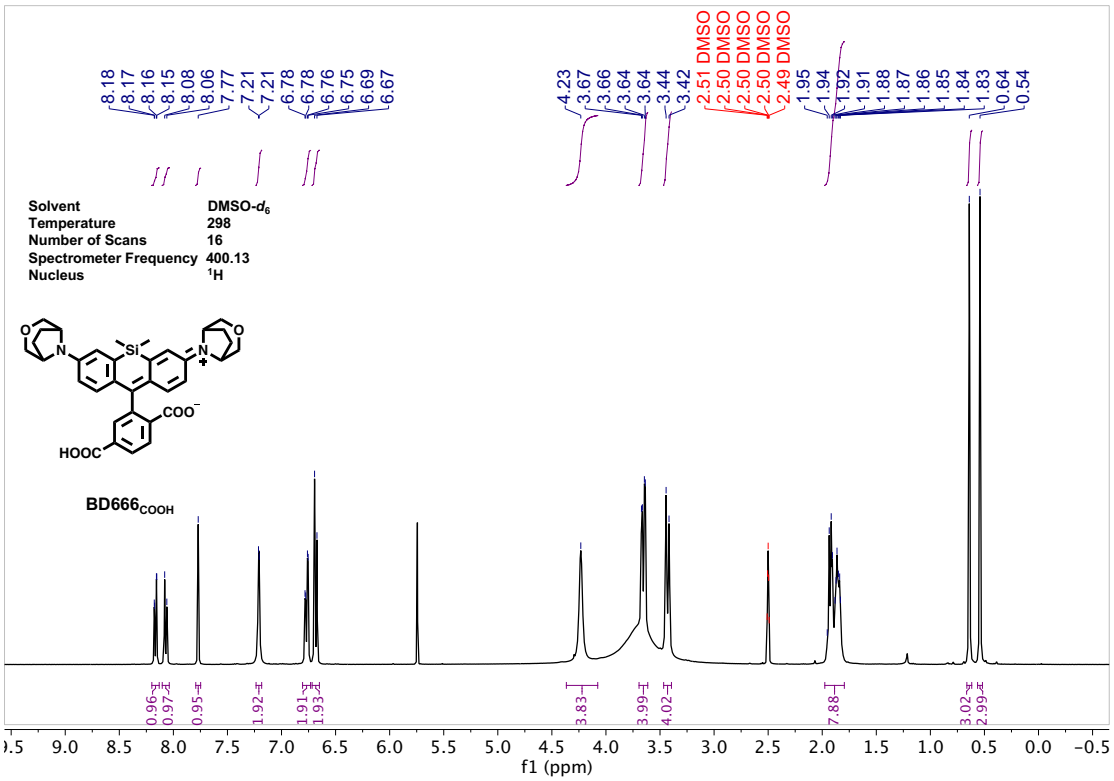

170.11
164.53
155.48
147.90
147.09
137.87
130.17
130.05
129.49
125.09
124.91
120.62
114.49
112.39
88.02
82.45
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
69.89
69.85
57.10
57.09
38.48
35.36
33.13
28.17
26.91
Solvent CDCl3
Temperature 298
Number of Scans 512
Spectrometer Frequency 100.61
Nucleus 13C
BD626COOt-Bu
f1 (ppm)

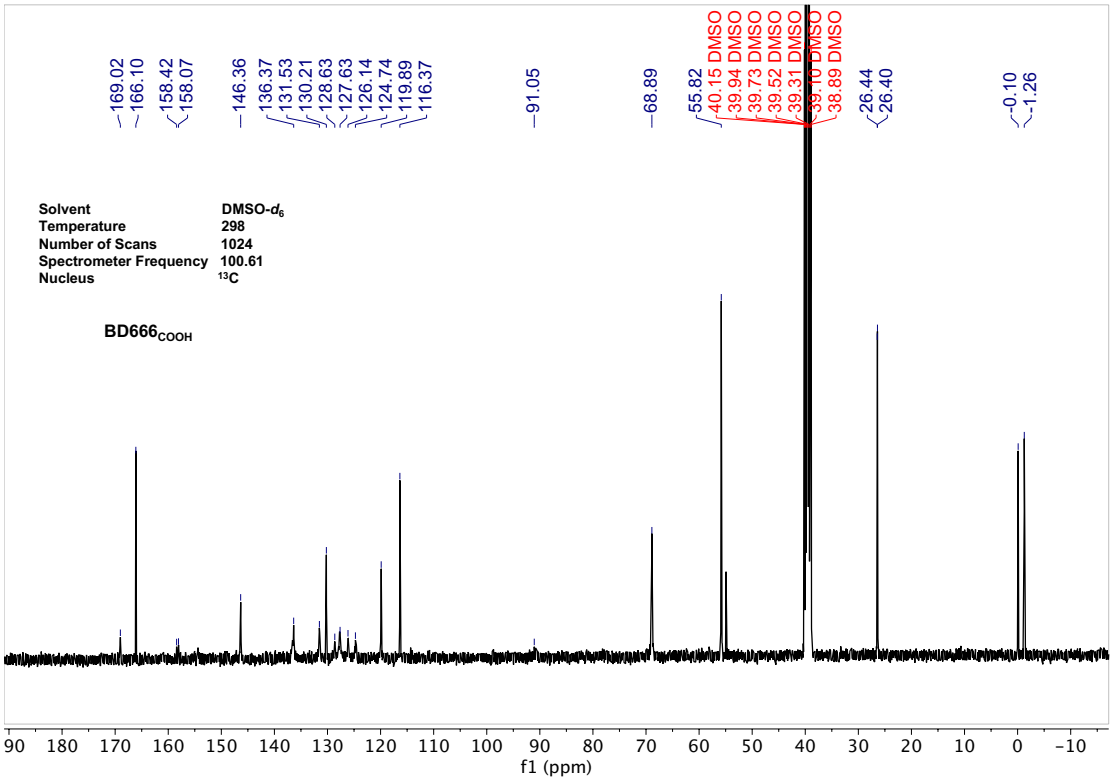

Solvent DMF-d_7
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus ^{1}H
HOOC
COO$^-$
BD626$_{COOH}$
f1 (ppm)

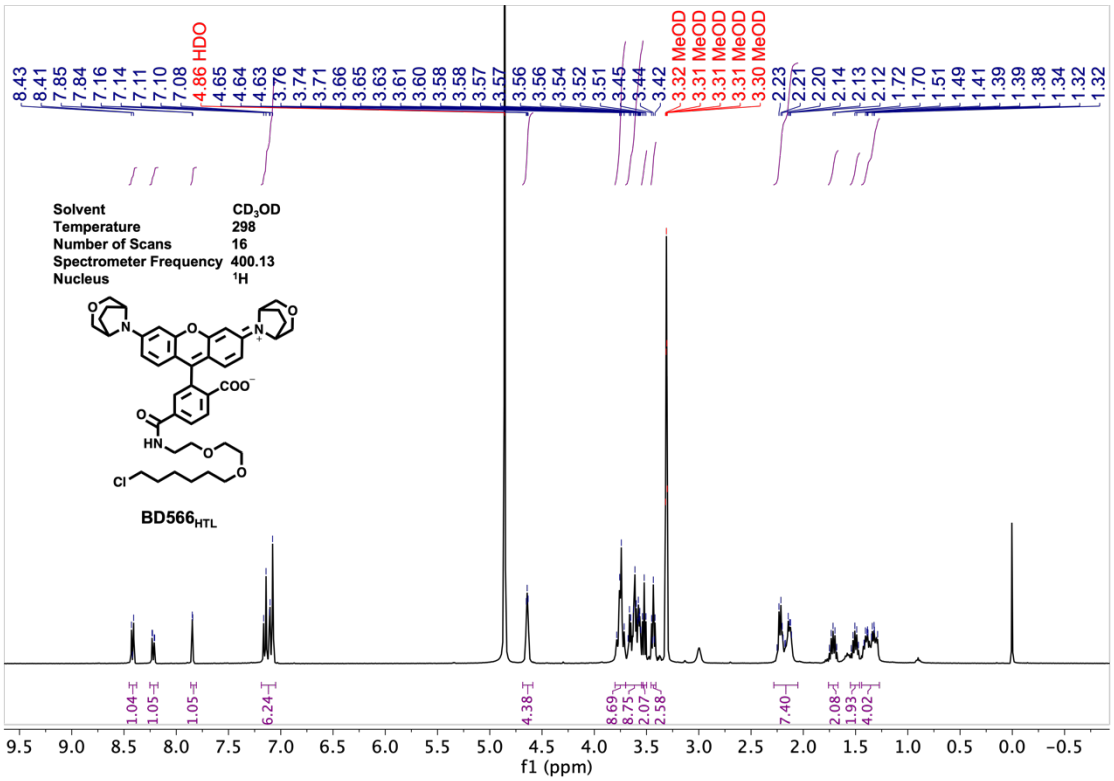

Response [mAU]
Retention time [min]
rel. Abundance [%]
Max: 599125
593.3
594.3
595.3
m/z

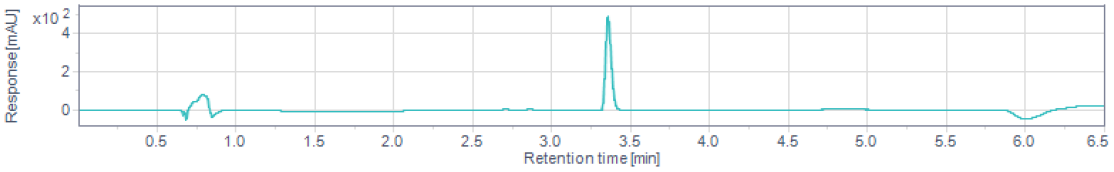

8.22
8.21
8.20
8.19
8.03
8.01
7.99
7.98
7.98
7.97
7.26 CDCl3
7.04
7.03
6.80
6.78
6.64
6.62
6.61
6.60
6.59
4.09
4.08
4.07
3.91
3.89
3.88
3.87
3.86
3.53
3.51
2.10
2.08
2.06
2.05
2.04
2.00
1.99
1.98
1.97
0.67
0.65
0.64
0.59
0.59
0.58
0.57
Solvent CDCl3
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
MeOOC
COO−
BD666COOMe
1.00
0.96
1.01
2.08
2.01
2.24
4.29
3.24
4.59
4.20
7.75
3.10
3.11
f1 (ppm)

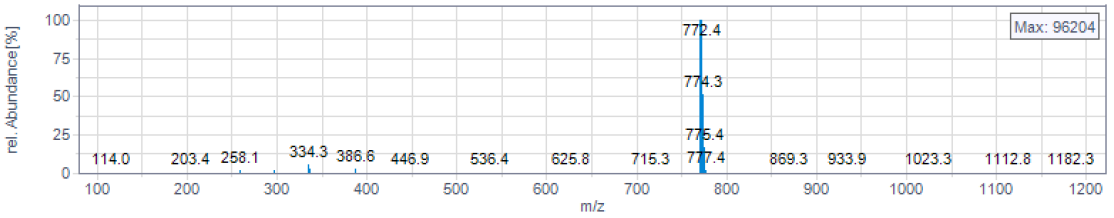

169.89
165.98
154.64
146.54
137.40
135.21
133.00
130.18
130.15
128.44
125.95
125.86
119.94
116.38
91.69
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
69.82
69.79
56.94
52.80
26.90
26.87
0.43
-1.22
Solvent CDCl3
Temperature 298
Number of Scans 1024
Spectrometer Frequency 100.61
Nucleus 13C
BD666COOMe
f1 (ppm)

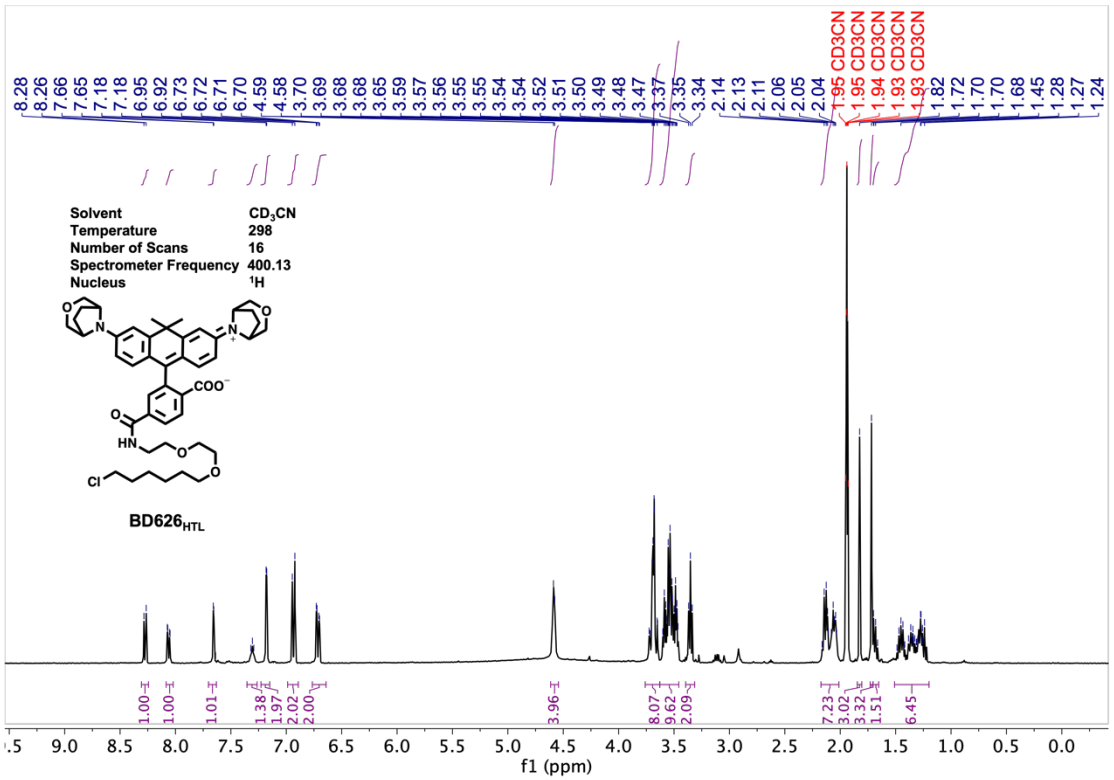

8.18
8.17
8.16
8.15
8.08
8.06
7.77
7.21
7.21
6.78
6.78
6.76
6.75
6.69
6.67
4.23
3.67
3.66
3.64
3.64
3.44
3.42
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.95
1.94
1.92
1.91
1.88
1.87
1.86
1.85
1.84
1.83
0.64
0.54
Solvent DMSO-d6
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
BD666COOH
0.96
0.97
0.95
1.92
1.91
1.93
3.83
3.99
4.02
7.88
3.02
2.99
f1 (ppm)

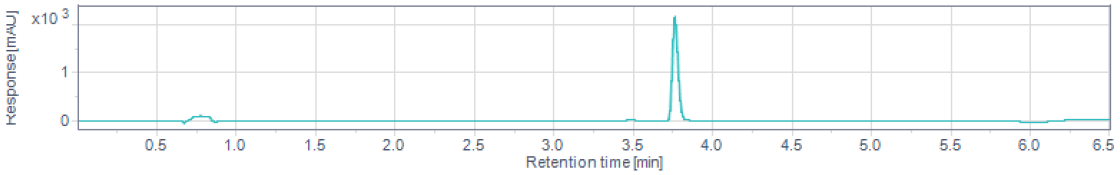

169.02
166.10
158.42
158.07
146.36
136.37
131.53
130.21
128.63
127.63
126.14
124.74
119.89
116.37
91.05
68.89
55.82
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.52 DMSO
39.31 DMSO
39.10 DMSO
38.89 DMSO
26.44
26.40
0.10
-1.26
Solvent DMSO-d6
Temperature 298
Number of Scans 1024
Spectrometer Frequency 100.61
Nucleus 13C
BD666COOH
f1 (ppm)

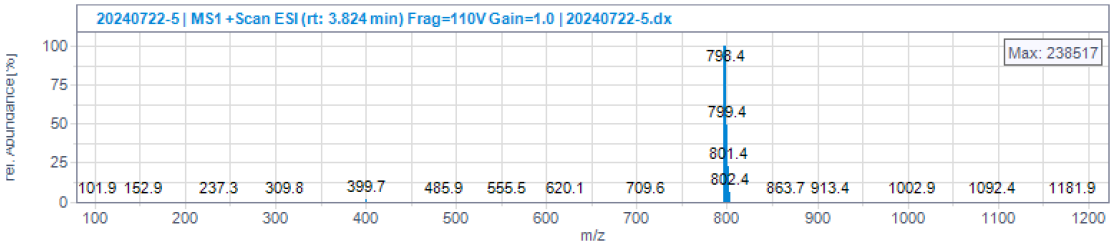

Solvent CD3OD
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
BD566HTL
f1 (ppm)

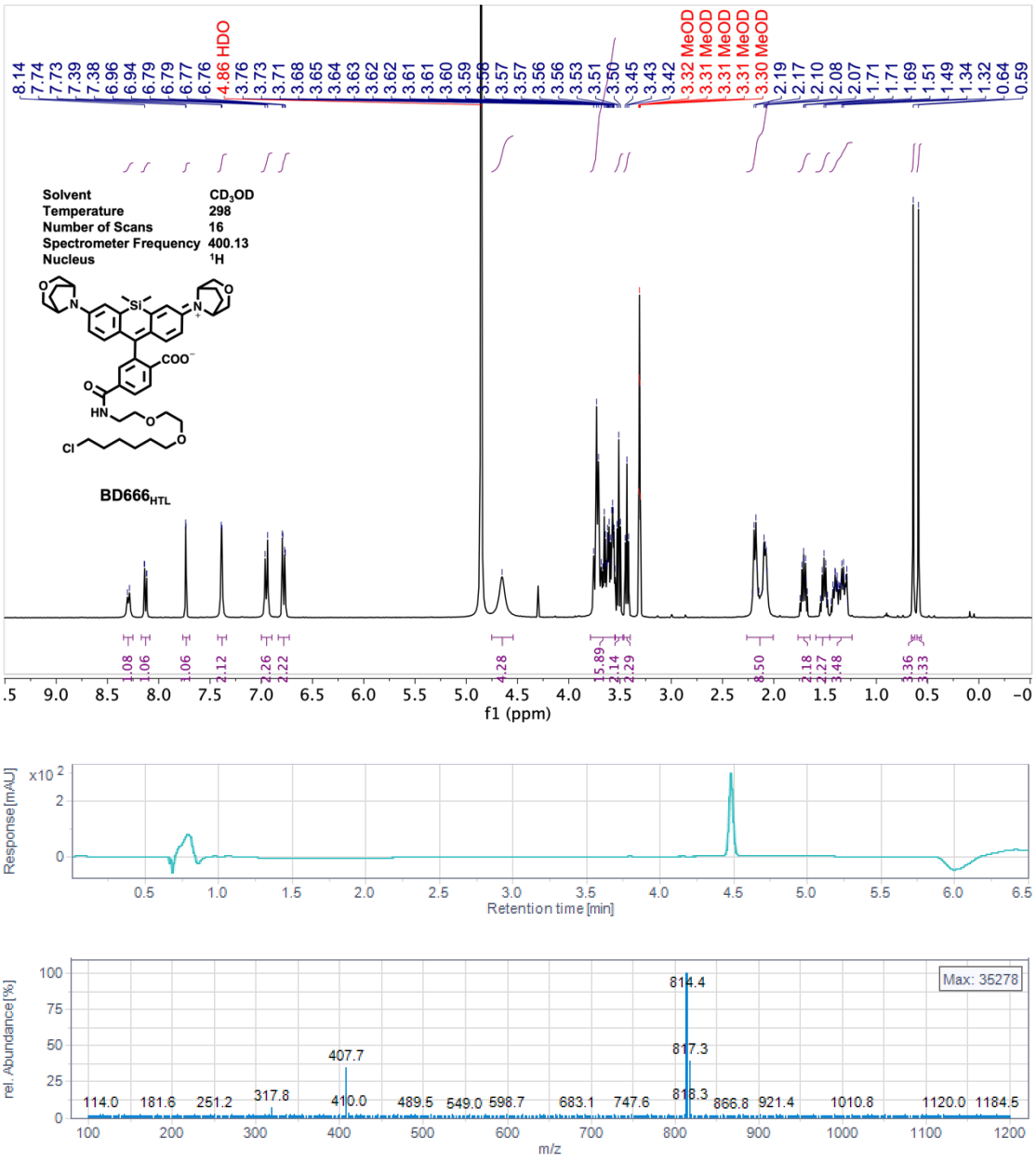

Response [mAU]
Retention time [min]


rel. Abundance [%]
Max: 96204
m/z


Solvent CD3CN
Temperature 298
Number of Scans 16
Spectrometer Frequency 400.13
Nucleus 1H
BD626HTL
f1 (ppm)


Response [mAU]
Retention time [min]


20240722-5 | MS1 +Scan ESI (rt: 3.824 min) Frag=110V Gain=1.0 | 20240722-5.dx
Max: 238517
798.4
799.4
801.4
802.4
rel. Abundance [%]
m/z



References for Supplementary Information:

1 Grimm, J., English, B., Chen, J. et al. A general method to improve fluorophores for live-cell and single-molecule microscopy. Nature Methods 12, 244–250 (2015).