nature portfolio

https://doi.org/10.1038/s41589-021-00775-w

Supplementary information

The HaloTag as a general scaffold for far-red tunable chemigenetic indicators

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SUPPLEMENTARY INFORMATION

The HaloTag as a general scaffold for far-red tunable chemigenetic indicators

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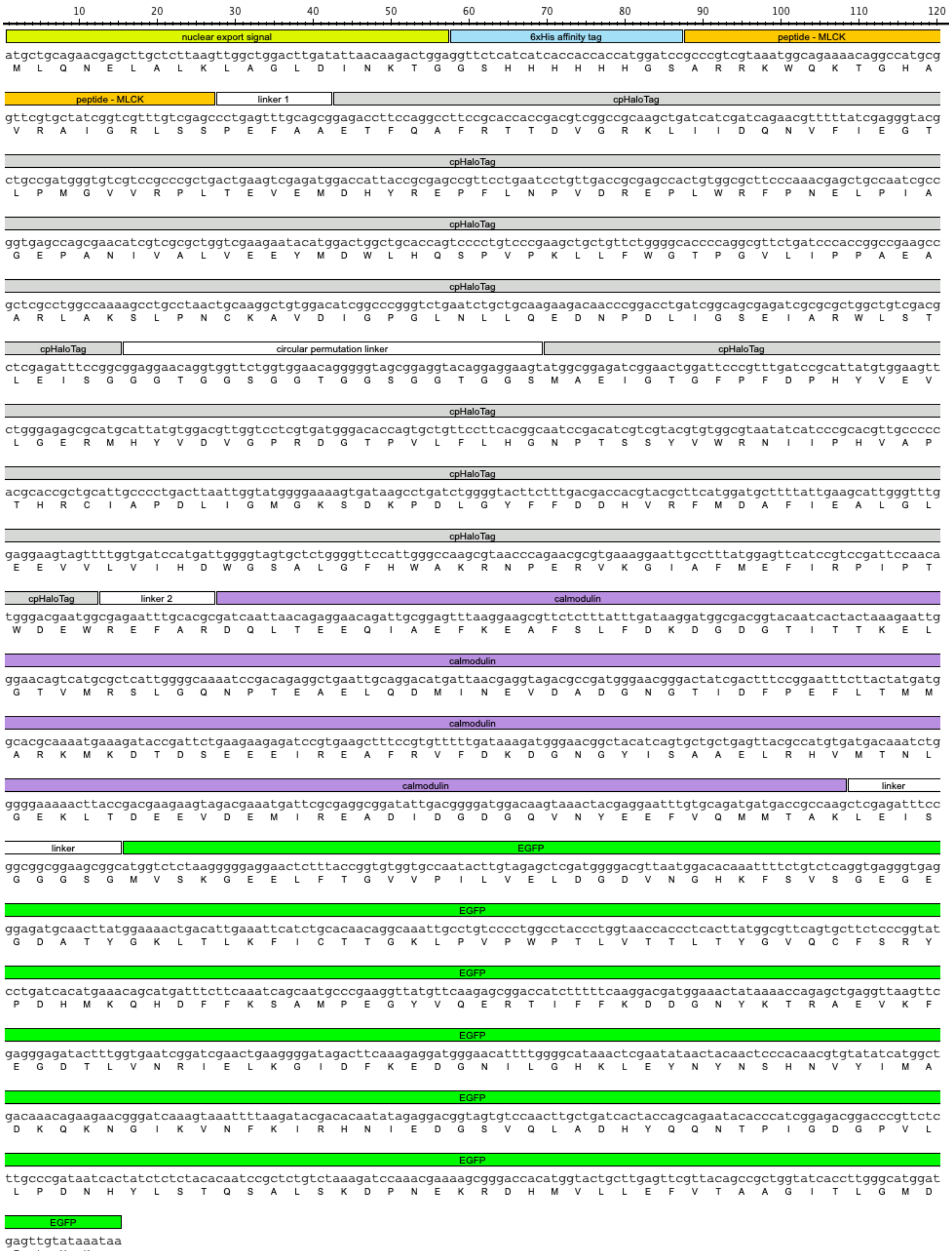
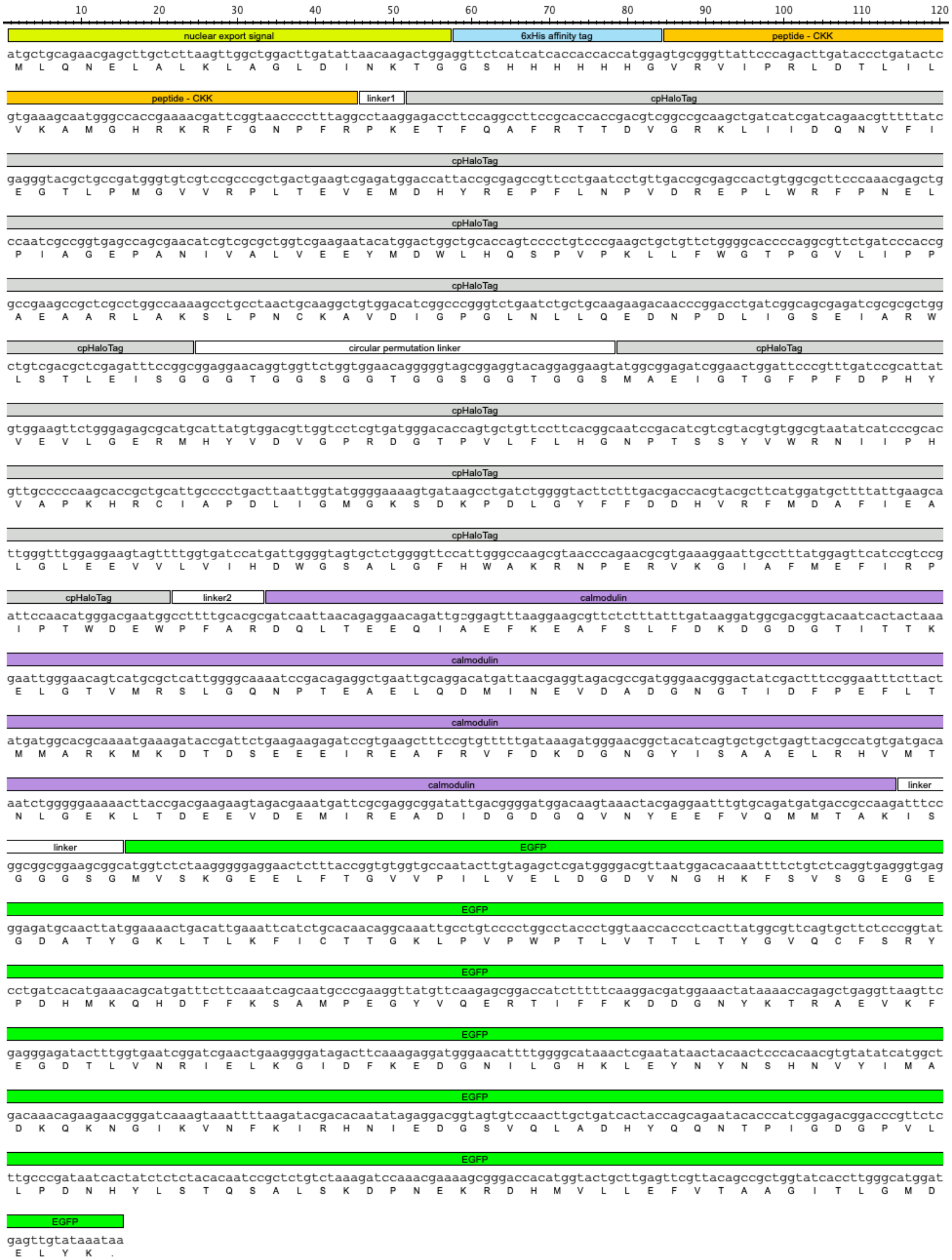
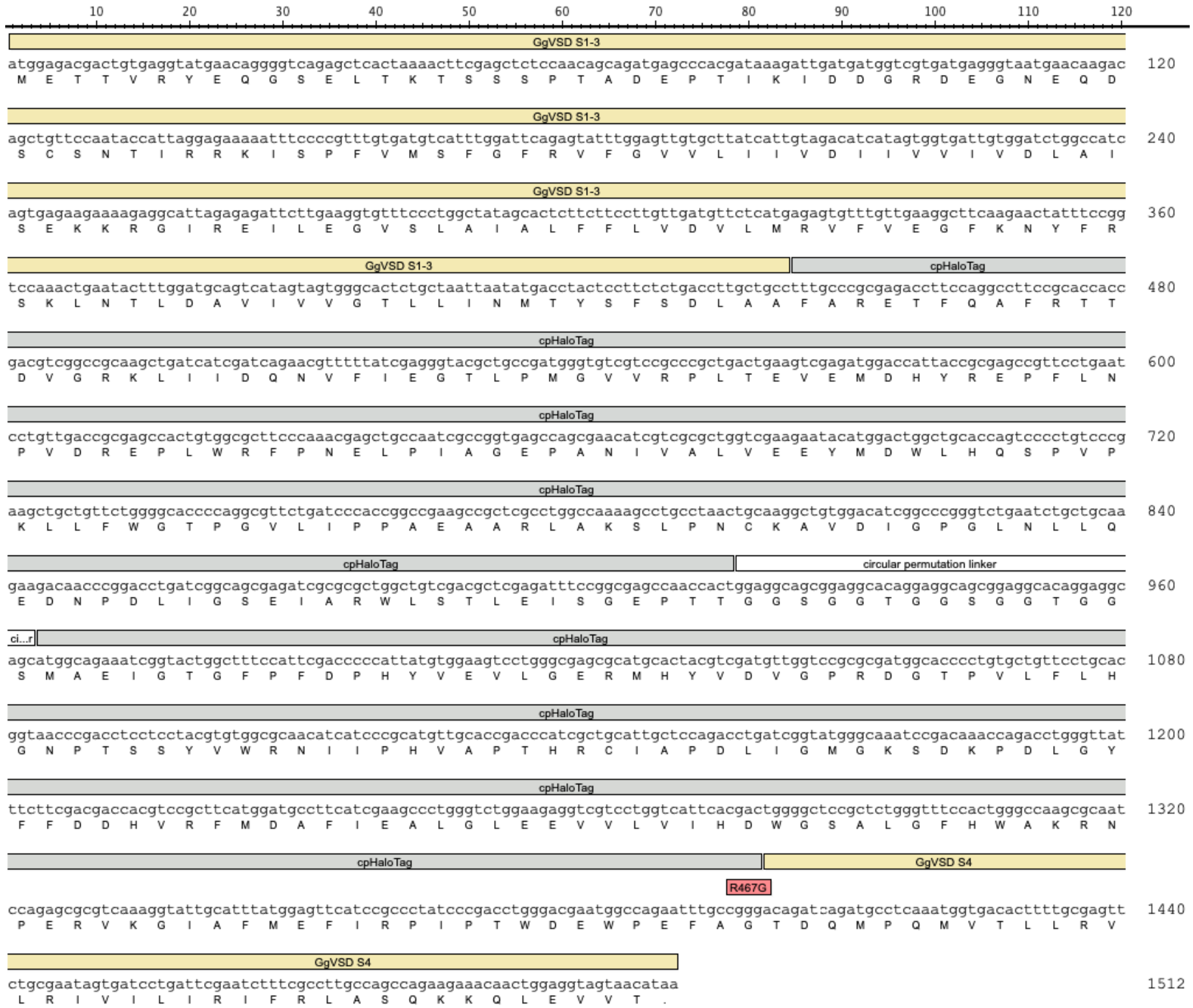
Supplementary Figure 1. DNA and amino acid sequences of HaloCaMP1a (a), HaloCaMP1b (b), HASAP1 (c), and HArcLight1 (d) annotated with sequence features.



a – HaloCaMP1a



b – HaloCaMP1b



c – HASAP1. At amino acid position 467, HASAP0.1=R, HASAP1=G.

```
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 M  E  G  F  D  G  S  D  F  S  P  P  A  D  L  V  G  V  G  G  A  V  M  R  N  V  V  D  V  T  I  N  G  D  V  T  A  P  P  K

CiVSD
gcagcgccaagaaaaagtgaatcggtaaagaaagttcattggaatgatgtagaccaaggaccgagtgaaaaaccagagacaagacaggaggaacgaatagatatacccgagatttcaggt
 A  A  P  R  K  S  E  S  V  K  K  V  H  W  N  D  V  D  Q  G  P  S  E  K  P  E  T  R  Q  E  E  R  I  D  I  P  E  I  S  G

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 L  W  W  G  E  N  E  H  G  V  G  G  G  R  M  E  I  P  T  T  G  V  G  R  V  Q  F  R  V  R  A  V  I  D  H  L  G  M  R  A

CiVSD
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 F  G  V  F  L  I  L  L  D  I  I  L  M  I  I  D  L  S  L  P  G  K  S  E  S  S  Q  S  F  Y  D  G  L  A  L  A  L  S  C  Y

CiVSD
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 F  M  L  D  L  G  L  R  I  F  A  Y  G  P  K  N  F  F  T  N  P  W  E  V  A  D  G  L  I  I  V  V  T  F  V  V  T  I  F  Y

CiVSD                                                                                                                  H...
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 T  V  L  D  E  Y  F  Q  E  T  G  A  D  G  L  G  Q  L  V  V  L  A  R  L  L  R  V  V  R  L  A  R  I  F  Y  S  H  Q  Q  I

HaloTag
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 G  T  G  F  P  F  D  P  H  Y  V  E  V  L  G  E  R  M  H  Y  V  D  V  G  P  R  D  G  T  P  V  L  F  L  H  G  N  P  T  S

HaloTag
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 S  Y  V  W  R  N  I  I  P  H  V  A  P  T  H  R  C  I  A  P  D  L  I  G  M  G  K  S  D  K  P  D  L  G  Y  F  F  D  D  H

HaloTag
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 V  R  F  M  D  A  F  I  E  A  L  G  L  E  E  V  V  L  V  I  H  D  W  G  S  A  L  G  F  H  W  A  K  R  N  P  E  R  V  K

HaloTag
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 G  I  A  F  M  E  F  I  R  P  I  P  T  W  D  E  W  P  E  F  A  R  E  T  F  Q  A  F  R  T  T  D  V  G  R  K  L  I  I  D

HaloTag
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 Q  N  V  F  I  E  G  T  L  P  M  G  V  V  R  P  L  T  E  V  E  M  D  H  Y  R  E  P  F  L  N  P  V  D  R  E  P  L  W  R

HaloTag
ttcccaaacgagctgccaatcgccggtgagccagcgaacatcgtcgcgctggtcgaagaatacatggactggctgcaccagtcccctgtcccgaagctgctgttctggggcaccccaggc
 F  P  N  E  L  P  I  A  G  E  P  A  N  I  V  A  L  V  E  E  Y  M  D  W  L  H  Q  S  P  V  P  K  L  L  F  W  G  T  P  G

HaloTag
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 V  L  I  P  P  A  E  A  A  R  L  A  K  S  L  P  N  C  K  A  V  D  I  G  P  G  L  N  L  L  Q  E  D  N  P  D  L  I  G  S

HaloTag
gagatcgcgcgctggctgtcgacgctcgagatttccggcgagccaaccacttaa
 E  I  A  R  W  L  S  T  L  E  I  S  G  E  P  T  T  .
```
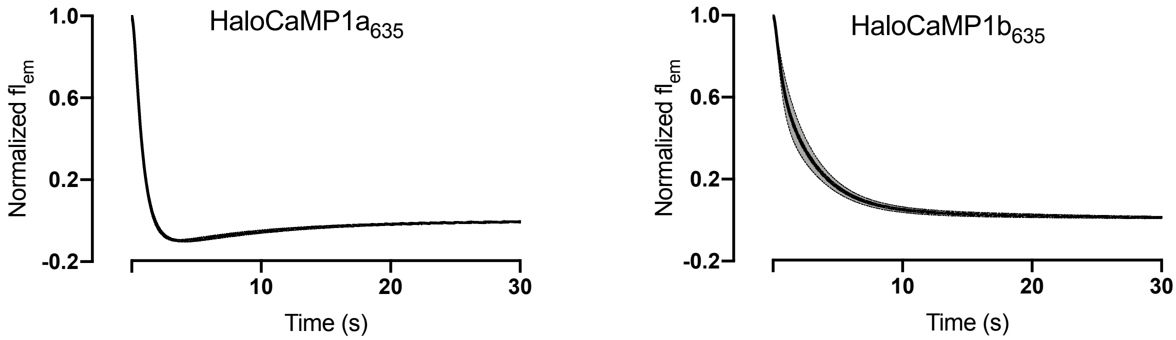
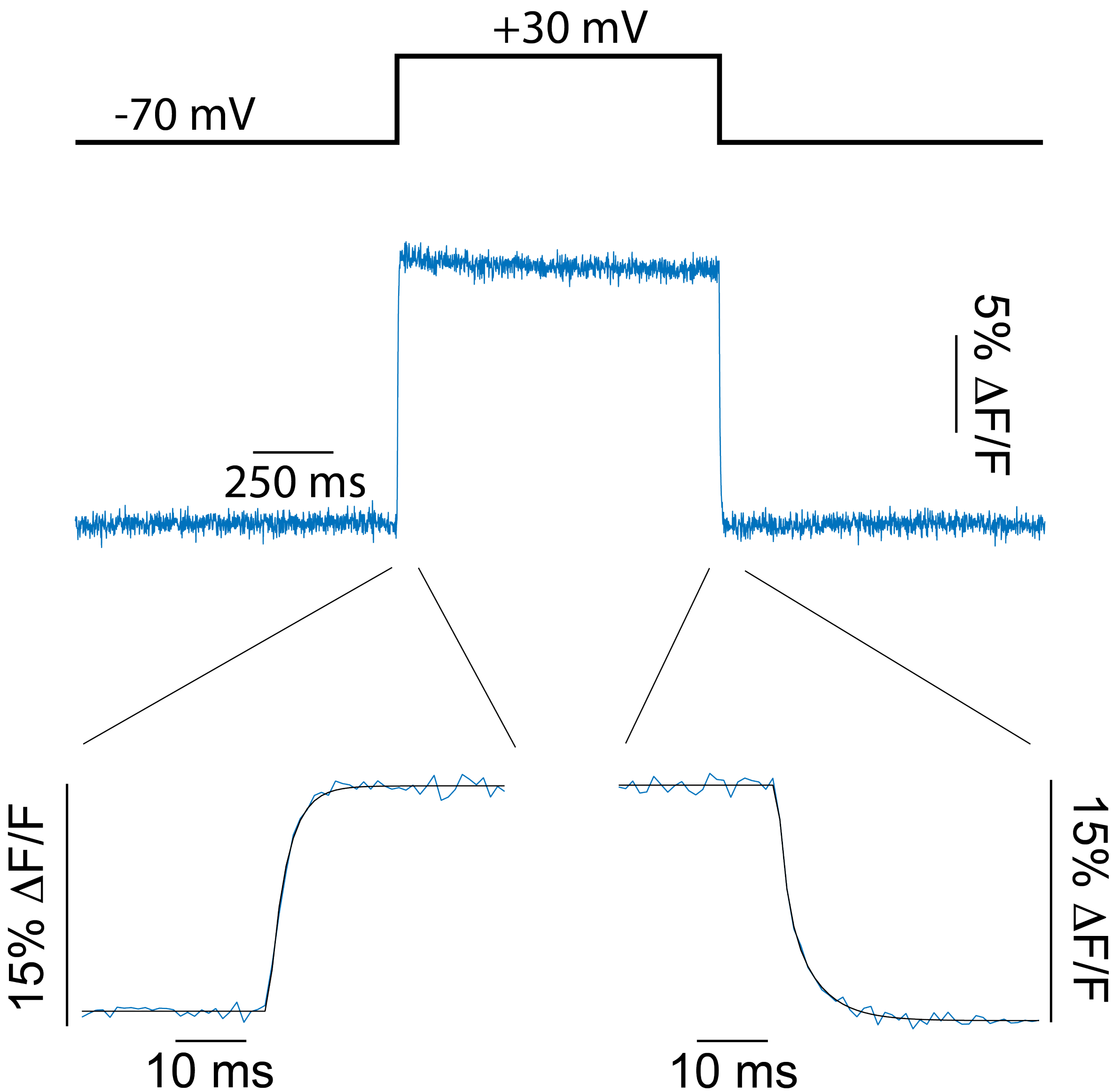

d – HArcLight1



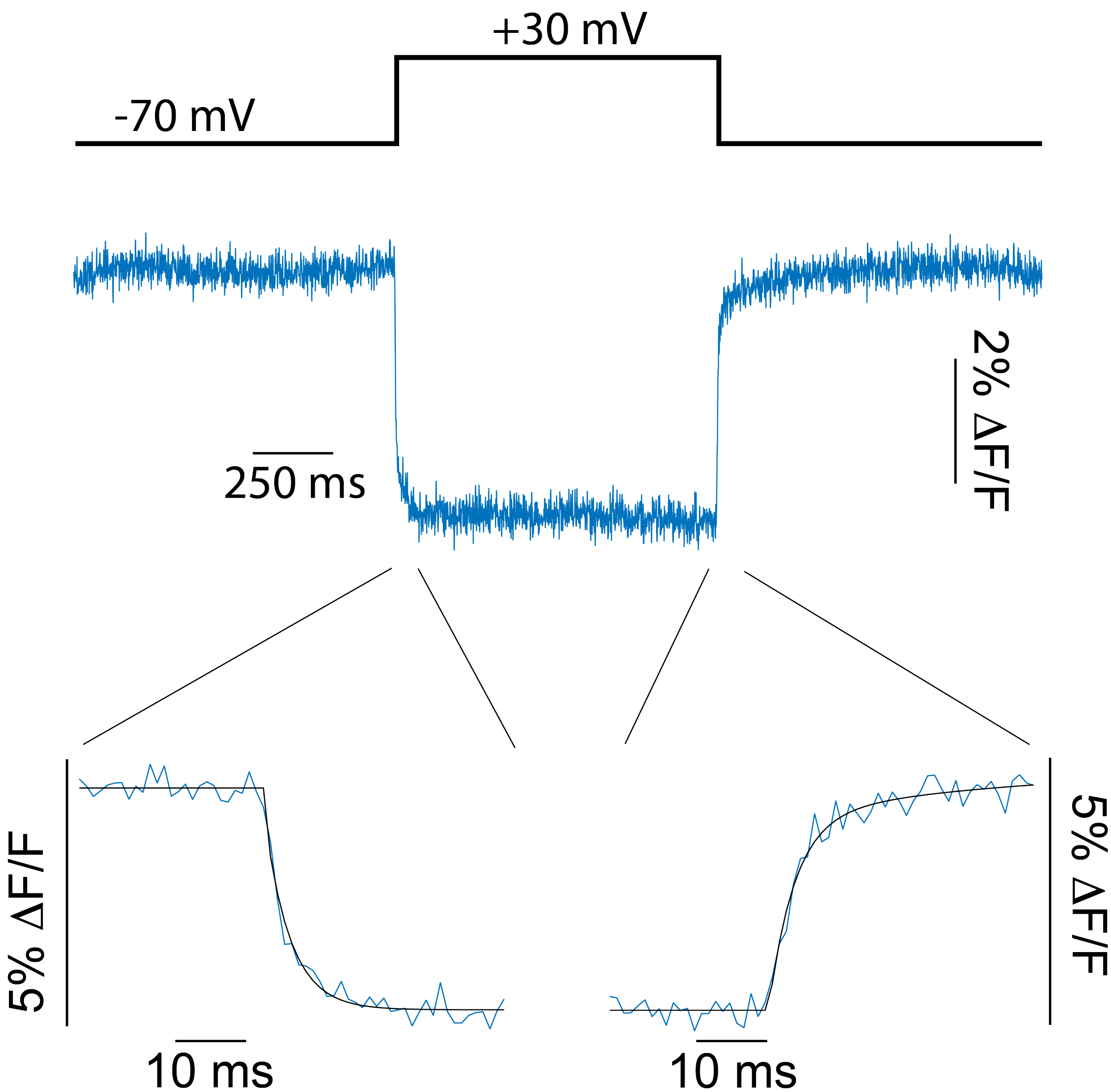
	HaloCaMP1a$_{635}$	HaloCaMP1b$_{635}$
Model	$y = a_0 + a_1(1 - e^{-b_1 \times t}) + a_2(1 - e^{-b_2 \times t})$	$y = a_0 + a_1(1 - e^{-b_1 \times t})$
fit	$y = 1 + (-1.2)(1 - e^{-0.99 \times t}) + (0.23)(1 - e^{-0.15 \times t})$	$y = 1 + (-0.99)(1 - e^{-0.43 \times t})$
k_{off} (s^{-1})	Fast, dissociative: 0.99, Slow, associative 0.15	Dissociative: 0.43

a_0 was constrained to 1.0.

Supplementary Figure 2. Kinetics of calcium unbinding from HaloCaMP1a or HaloCaMP1b bound to JF_{635}-HTL. A stopped flow instrument was used follow the decrease in fluorescence emission from recombinant calcium-saturated HaloCaMP$_{635}$ following rapid mixing with excess calcium chelator (EGTA, 10 mM). HaloCaMP1a was fit to a two-phase exponential model and HaloCaMP1b was fit to a one-phase exponential model. Mean and s.d. for 27 trials over 3 independent days, normalized to the initial fluorescence intensity at time 0.



Supplementary Figure 3. Fluorescence response of HASAP1$_{635}$ in response to a 100 mV potential step. Insets: Zoom in on fluorescence response to membrane depolarization (from -70 mV to +30 mV), and repolarization (from +30 mV to -70 mV). Solid black line is fit of rise and decay kinetics to a double exponential function. Image acquisition rate 1200 Hz. See Table S4 for full kinetic data.



Supplementary Figure 4. Fluorescence response of HArclight1$_{635}$ in response to a 100 mV potential step. Insets: Zoom in on fluorescence response to membrane depolarization (from -70 mV to +30 mV), and repolarization (from +30 mV to -70 mV). Solid black line is fit of rise and decay kinetics to a double exponential function. Image acquisition rate 1200 Hz. See Table S4 for full kinetic data.

SUPPLEMENTARY TABLES

	HaloTag-TMR (PDB 6U32)	Ca^{2+}-HaloCaMP1b-JF_{635} (PDB 6U2M)
Data collection		
Space group	$P4_32_12$	P2
Cell dimensions		
a, *b*, *c* (Å)	62.53, 62.53, 164.17	92.56, 60.66, 122.60
α, β, γ(°)	90, 90, 90	90, 91.0, 90
Resolution (Å)	62.53 – 1.80 (1.90 – 1.80)*	92.54 – 2.00 (2.11 – 2.00)
R_{sym} (%)	10.3 (54.5)	9.6 (69.6)
$I / \sigma I$	11.2 (2.4)	6.5 (1.1)
Completeness (%)	97.0 (97.6)	98.6 (97.6)
Redundancy	5.8 (5.9)	4.6 (4.4)
Refinement		
Resolution (Å)	58.43 – 1.80	122.58 – 2.00
No. reflections	30,022	90,798
R_{work} / R_{free}	15.7/19.3	18.8/22.6
No. atoms		
Protein	2350	7420
Dye-HaloTag ligand	76	100
Chloride ions	1	2
Calcium ions	-	8
Water	177	203
B-factors		
Protein	28.8	53.1
Dye-HaloTag ligand	37.9	87.9
Chloride ions	20.8	38.9
Calcium ions	-	54.0
Water	36.4	47.8
R.m.s. deviations		
Bond lengths (Å)	0.030	0.026
Bond angles (°)	2.54	2.44

Supplementary Table 1. X-ray diffraction data collection and model refinement statistics. *Values in parentheses are for highest-resolution shell. One crystal was used for each structure.

HaloCaMP variant	Peptide	L1	L2	ε_{sat} ($M^{-1}.cm^{-1}$)	Φ_{sat}	Brightness ($mM^{-1}.cm^{-1}$)	$\Delta F/F_0$	K_d (nM)
1a	MLCK	PEFAA	REFAR	96,000	0.78	74.9	5.0	190
1b	CKK	PK	PFAR	60,000	0.75	45.0	9.2	43

Supplementary Table 2. Properties of HaloCaMP variants 1a and 1b labeled with JF_{635}-HaloTag ligand.

Dye	λ_{ex} (nm)	λ_{em} (nm)	ε ($M^{-1}.cm^{-1}$)	Φ
JF_{635}	635	652	~400	0.56
JF_{646}	646	664	5000	0.54
JF_{639}	639	656	5000	0.62
JF_{630}	630	649	~700	NM
JF_{629}	629	648	<200	NM
JF_{626}	626	638	<200	NM
JF_{614}	614	631	<200	NM

Supplementary Table 3. Photophysical properties of azetidine-substituted Si-rhodamines in 10 mM HEPES, pH = 7.4. NM: not measured.

Ligand		λ_{ex} (nm)	λ_{em} (nm)	ε ($M^{-1}.cm^{-1}$)	Φ
1 (JF$_{635}$-HaloTag ligand)[9]	− HaloTag	635	652	~400	NM
	+ HaloTag	640	656	81000	0.75
5 (JF$_{646}$-HaloTag ligand)[9]	− HaloTag	649	666	6000	0.52
	+ HaloTag	652	666	95000	0.64
6 (JF$_{639}$-HaloTag ligand)	− HaloTag	645	658	5300	0.63
	+ HaloTag	647	663	120000	0.71
7 (JF$_{630}$-HaloTag ligand)	− HaloTag	633	657	1200	NM
	+ HaloTag	639	656	32000	0.70
8 (JF$_{629}$-HaloTag ligand)	− HaloTag	638	655	<200	NM
	+ HaloTag	638	656	29000	0.81
9 (JF$_{626}$-HaloTag ligand)	− HaloTag	634	647	<200	NM
	+ HaloTag	639	654	57000	0.73
10 (JF$_{614}$-HaloTag ligand)	− HaloTag	622	640	<200	NM
	+ HaloTag	628	646	7000	0.74

Supplementary Table 4. Photophysical properties of Si-rhodamines HaloTag ligands in the presence or absence of HaloTag protein in 10 mM HEPES pH = 7.4 containing 0.1 mg·mL^{-1} CHAPS. NM: not measured.

	HaloCaMP1a		**HaloCaMP1b**	
Ligand	**$\Delta F/F_0$**	**K_d (nM)**	**$\Delta F/F_0$**	**K_d (nM)**
1 (JF$_{635}$-HaloTag ligand)[9]	5.0	190	9.2	43
5 (JF$_{646}$-HaloTag ligand)[9]	0.6	65	0.9	19
6 (JF$_{639}$-HaloTag ligand)	1.1	128	2.3	32
7 (JF$_{630}$-HaloTag ligand)	7.8	340	8.7	67
8 (JF$_{629}$-HaloTag ligand)	13.8	118	20.9	44
9 (JF$_{626}$-HaloTag ligand)	11.8	391	7.4	42
10 (JF$_{614}$-HaloTag ligand)	29.5	892	10.8	61

Supplementary Table 5. Ca^{2+} binding properties of HaloCaMP1a and 1b bound to Si-rhodamine ligands measured in solution.

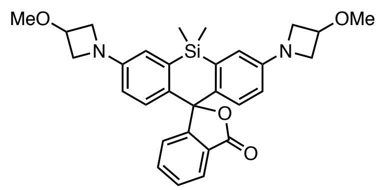
	Activation (-70 mV to 30 mV)			Deactivation (30 mV to -70 mV)		
	τ_{fast} (ms)	τ_{slow} (ms)	% fast	τ_{fast} (ms)	τ_{slow} (ms)	% fast
HASAP1-JF_{635}	2.1 ± 0.2	5.2 ± 0.6	96 ± 3	1.1 ± 0.1	3.7 ± 0.3	50 ± 8
HArclight1-JF_{635}	2.2 ± 0.2	8.5 ± 0.3	54 ± 5	1.6 ± 0.2	8.1 ± 0.6	37 ± 3

Supplementary Table 6. HASAP1 and HArclight1 kinetics in primary rat neuron cultures. Neurons expressing HASAP1 and Harclight1 were imaged at 1 kHz during whole cell voltage clamp. Fluorescence traces were fit using a double exponential function (Supplementary Figs. 12,14). % fast is the percentage of fluorescence change attributed to the fast-changing component of the bi-exponential fit to the fluorescence change. The remainder is attributed to the slow-changing component. Errors are s.e.m. N = 8 cells for HASAP1 and N = 6 cells for HArclight1.

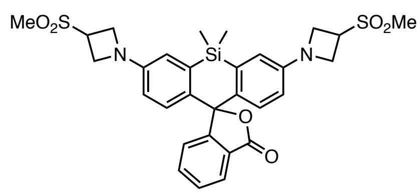
SUPPLEMENTARY NOTE

SYNTHETIC PROCEDURES

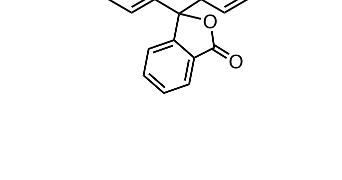
Procedure A: Synthesis of Si-rhodamines by Pd-catalyzed cross-coupling. The following procedure for (**12**; **JF$_{639}$**) is representative. A vial was charged with silafluorescein ditriflate **11**[10] (50 mg, 78 µmol), 3-methoxyazetidine hydrochloride (39 mg, 312 µmol, 4 eq), Pd_2dba_3 (7.1 mg, 7.8 µmol, 0.1 eq), XPhos (11.2 mg, 23.4 µmol, 0.3 eq), and Cs_2CO_3 (204 mg, 625 mmol, 8 eq). The vial was sealed and evacuated/backfilled with nitrogen (3x). Dioxane (2 mL) was added, and the reaction was flushed again with nitrogen (3x). The reaction was then stirred at 100 °C overnight. It was subsequently cooled to room temperature, diluted with MeOH, deposited onto Celite, and concentrated to dryness. The residue was purified as described.



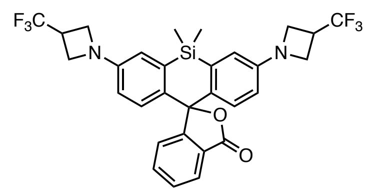
(12; JF$_{639}$): Purification by silica gel chromatography (0–35% EtOAc/toluene, linear gradient) afforded **12** (78%) as a light blue solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.96 (d, J = 7.6 Hz, 1H), 7.64 (td, J = 7.5, 1.2 Hz, 1H), 7.54 (td, J = 7.5, 1.0 Hz, 1H), 7.32 – 7.27 (m, 1H), 6.77 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 2.7 Hz, 2H), 6.28 (dd, J = 8.7, 2.7 Hz, 2H), 4.38 – 4.27 (m, 2H), 4.10 (d, J = 7.3 Hz, 4H), 3.73 (dt, J = 7.7, 4.0 Hz, 4H), 3.32 (s, 6H), 0.61 (s, 3H), 0.58 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.7 (C), 154.3 (C), 150.4 (C), 137.1 (C), 133.8 (CH), 133.3 (C), 128.9 (CH), 128.1 (CH), 127.0 (C), 125.9 (CH), 124.7 (CH), 116.1 (CH), 112.7 (CH), 91.9 (C), 70.1 (CH_3), 58.9 (CH_2), 56.2 (CH), 0.5 (CH_3), -1.4 (CH_3); HRMS (ESI) calcd for $C_{30}H_{33}N_2O_4Si$ $[M+H]^+$ 513.2210, found 513.2202.



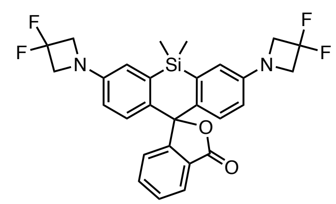
(13; JF$_{630}$): Synthesized following procedure A from silafluorescein ditriflate and 3-methylsulfonyl-azetidine hydrochloride. Purification by silica gel chromatography (50–100% EtOAc/hexane, linear gradient) afforded **13** (80%) as a light blue solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.96 (dt, J = 7.6, 1.0 Hz, 1H), 7.65 (td, J = 7.5, 1.2 Hz, 1H), 7.55 (td, J = 7.5, 1.0 Hz, 1H), 7.28 – 7.25 (m, 1H), 6.83 (d, J = 8.7 Hz, 2H), 6.70 (d, J = 2.7 Hz, 2H), 6.31 (dd, J = 8.7, 2.7 Hz, 2H), 4.29 – 4.15 (m, 8H), 4.07 (tt, J = 7.5, 5.7 Hz, 2H), 2.96 (s, 6H), 0.62 (s, 3H), 0.59 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.6 (C), 154.2 (C), 149.2 (C), 137.1 (C), 134.8 (C), 134.1 (CH), 129.1 (CH), 128.2 (CH), 126.6 (C), 126.0 (CH), 124.6 (CH), 116.2 (CH), 113.0 (CH), 91.2 (C), 52.5 (CH_2), 52.4 (CH_2), 51.7 (CH), 38.3 (CH_3), 0.4 (CH_3), -1.3 (CH_3); HRMS (ESI) calcd for $C_{30}H_{33}N_2O_6SiS_2$ $[M+H]^+$ 609.1549, found 609.1548.



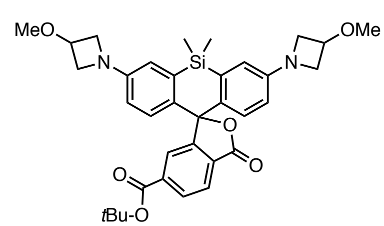
(14; JF_{629}): Synthesized following procedure A from silafluorescein ditriflate and 3-azetidinecarbonitrile hydrochloride. Purification by HPLC (35–95% $MeCN/H_2O$ + 0.1% TFA additive) afforded **14** (42%) as a light blue solid. ^1H NMR (CD_2Cl_2, 400 MHz) δ 7.93 (dt, *J* = 7.6, 1.0 Hz, 1H), 7.66 (td, *J* = 7.5, 1.2 Hz, 1H), 7.57 (td, *J* = 7.5, 1.0 Hz, 1H), 7.24 (dd, *J* = 7.7, 1.0 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.72 (d, *J* = 2.7 Hz, 2H), 6.33 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.20 (ddd, *J* = 8.6, 7.1, 1.8 Hz, 4H), 4.13 – 4.03 (m, 4H), 3.60 (tt, *J* = 8.4, 6.1 Hz, 2H), 0.63 (s, 3H), 0.57 (s, 3H); ^{13}C NMR (CD_2Cl_2, 101 MHz) δ ^{13}C NMR (101 MHz, CD_2Cl_2) δ 170.7 (C), 154.8 (C), 150.1 (C), 137.3 (C), 135.0 (C), 134.6 (CH), 129.6 (CH), 128.5 (CH), 126.8 (C), 126.3 (CH), 124.8 (CH), 120.5 (C), 116.6 (CH), 113.4 (CH), 91.4 (C), 55.9 (CH_2), 19.1 (CH), 0.4 (CH_3), -1.1 (CH_3); HRMS (ESI) calcd for $C_{30}H_{27}N_4O_2Si$ $[M+H]^+$ 503.1903, found 503.1899.



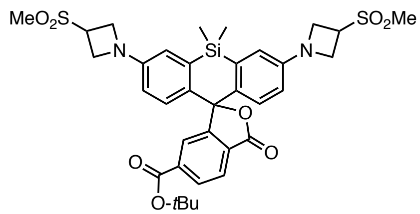
(15; JF_{626}): Synthesized following procedure A from silafluorescein ditriflate and 3-(trifluoromethyl)azetidine hydrochloride. Purification by silica gel chromatography (0–100% EtOAc/hexane, linear gradient), followed by purification by silica gel chromatography (0–35% EtOAc/toluene) afforded **15** (76%) as a light blue solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.97 (dt, *J* = 7.7, 1.0 Hz, 1H), 7.66 (td, *J* = 7.5, 1.2 Hz, 1H), 7.56 (td, *J* = 7.5, 1.0 Hz, 1H), 7.29 (dd, *J* = 7.7, 0.9 Hz, 1H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.70 (d, *J* = 2.7 Hz, 2H), 6.29 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.14 – 4.04 (m, 4H), 4.01 – 3.91 (m, 4H), 3.49 – 3.29 (m, 2H), 0.62 (s, 3H), 0.60 (s, 3H); ^{19}F NMR ($CDCl_3$, 376 MHz) = -73.4 (d, $^3J_{HF}$ = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.5 (C), 154.1 (C), 149.5 (C), 137.2 (C), 134.1 (CH), 133.9 (C), 129.1 (CH), 128.2 (CH), 126.5 (q, $^2J_{CF}$ = 81.5Hz, CF_3), 126.0 (CH), 124.7 (CH), 115.8 (CH), 112.5 (CH), 91.5 (C), 51.4 (CH_2), 51.3 (CH2), 32.8 (q, $^3J_{CF}$ = 32.3 Hz, C), 0.5 (CH_3), -1.5 (CH_3); HRMS (ESI) calcd for $C_{30}H_{27}N_2O_2SiF_6$ $[M+H]^+$ 589.1746, found 589.1751.



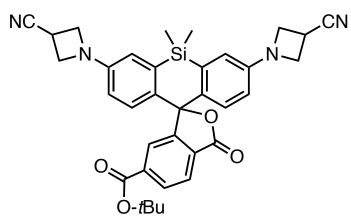
(16; JF_{614}): Synthesized following procedure A from silafluorescein ditriflate and 3,3-difluoroazetidine hydrochloride. Purification by silica gel chromatography (0–100% EtOAc/hexane, linear gradient), followed by purification by silica gel chromatography (0–35% EtOAc/toluene) afforded **16** (24%) as a light blue solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.98 (dt, *J* = 7.6, 1.0 Hz, 1H), 7.66 (td, *J* = 7.5, 1.2 Hz, 1H), 7.56 (td, *J* = 7.5, 1.0 Hz, 1H), 7.30 – 7.28 (m, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 6.73 (d, *J* = 2.7 Hz, 2H), 6.34 (dd, *J* = 8.7, 2.8 Hz, 2H), 4.23 (t, $^3J_{HF}$ = 11.8 Hz, 8H), 0.64 (s, 3H), 0.61 (s, 3H); ^{19}F NMR ($CDCl_3$, 376 MHz) = -99.9 (p, $^3J_{HF}$ = 11.7 Hz) ;^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.5 (C), 154.0 (C), 148.7 (t, $^4J_{CF}$ = 2.6 Hz, C), 137.3 (C), 134.8 (C), 134.0 (CH), 129.2 (CH), 128.2 (CH), 126.8 (C), 126.1 (CH), 124.6 (CH), 116.8 (CH), 115.9 (t, $^1J_{CF}$ = 276 Hz, CF_2) , 113.6 (CH), 91.2 (C), 63.4 (t, $^2J_{HF}$ = 25.9,CH_2), 0.4 (CH_3), -1.4 (CH_3); HRMS (ESI) calcd for $C_{28}H_{25}N_2O_2SiF_4$ $[M+H]^+$ 525.1621, found 525.1629.



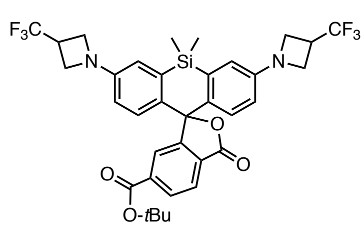
(17): Synthesized following procedure A from 6-*tert*-butoxycarbonylsilafluorescein ditriflate **4**[10] and 3-methoxyazetidine hydrochloride. Purification by silica gel chromatography (0–30% EtOAc/hexane, linear gradient), afforded **17** (94%) as an off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 1.2 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 6.68 (d, *J* = 2.7 Hz, 2H), 6.32 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.38 – 4.27 (m, 2H), 4.16 – 4.04 (m, 4H), 3.78 – 3.68 (m, 4H), 3.32 (s, 6H), 1.55 (s, 9H), 0.65 (s, 3H), 0.58 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.3 (C), 164.4 (C), 155.4 (C), 150.4 (C), 137.3 (C), 136.2 (C), 132.8 (C), 130.0 (CH), 129.1 (C), 127.7 (CH), 125.7 (CH), 125.1 (CH), 116.1 (CH), 113.1 (CH), 91.7 (C), 82.4 (C), 70.1 (CH_3), 58.9 (CH_2), 56.2 (CH), 28.2 (CH_3), 0.2 (CH_3), -0.7 (CH_3); HRMS (ESI) calcd for $C_{35}H_{41}N_2O_6Si$ $[M+H]^+$ 613.2734, found 613.2726.



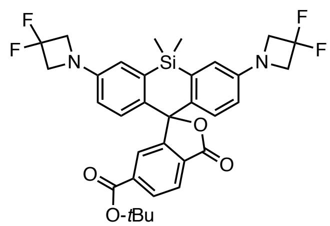
(18): Synthesized following procedure A from 6-*tert*-butoxycarbonylsilafluorescein ditriflate **4** and 3-methylsulfonyl-azetidine hydrochloride. Purification by silica gel chromatography (50–100% EtOAc/hexane, linear gradient), afforded **18** (87%) as a light blue solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.12 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.97 (dd, *J* = 8.0, 0.7 Hz, 1H), 7.79 (t, *J* = 1.0 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.72 (d, *J* = 2.7 Hz, 2H), 6.38 (dd, *J* = 8.8, 2.7 Hz, 2H), 4.31 – 4.19 (m, 8H), 4.15 – 4.03 (m, 2H), 2.97 (s, 6H), 1.55 (s, 9H), 0.67 (s, 3H), 0.59 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.2 (C), 164.3 (C), 155.2 (C), 149.2 (C), 137.5 (C), 136.2 (C), 134.3 (C), 130.2 (CH), 128.7 (C), 127.8 (CH), 125.9 (CH), 124.9 (CH), 116.2 (CH), 113.3 (CH), 90.9 (C), 82.6 (C), 52.5 (CH_2), 51.7 (CH), 38.3 (CH_3), 28.2 (CH_3), 0.1 (CH_3), -0.6 (CH_3); HRMS (ESI) calcd for $C_{35}H_{41}N_2O_8SiS_2$ $[M+H]^+$ 709.2073, found 709.2074.



(19): Synthesized following procedure A from 6-*tert*-butoxycarbonylsilafluorescein ditriflate **4** and 3-azetidinecarbonitrile hydrochloride. Purification by silica gel chromatography (0–20% EtOAc/hexane, linear gradient) afforded **19** (88%) as a light blue solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.97 (dd, *J* = 8.0, 0.7 Hz, 1H), 7.81 (t, *J* = 1.0 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.68 (d, *J* = 2.7 Hz, 2H), 6.33 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.25 – 4.17 (m, 4H), 4.14 – 4.02 (m, 4H), 3.59 (tt, *J* = 8.4, 6.2 Hz, 2H), 1.55 (s, 9H), 0.68 (s, 3H), 0.59 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) 170.0 (C), 164.3 (C), 154.9 (C), 149.4 (C), 137.5 (C), 136.3 (C), 134.4 (C), 130.2 (CH), 128.8 (C), 127.8 (CH), 125.9 (CH), 124.9 (CH), 119.7 (C), 116.1 (CH), 113.3 (CH), 90.9 (C), 82.6 (C), 55.3 (CH_2), 28.2 (CH_3), 18.5 (CH), 0.1 (CH_3), -0.7 (CH_3); HRMS (ESI) calcd for $C_{35}H_{35}N_4O_4Si$ $[M+H]^+$ 603.2428, found 603.2425.

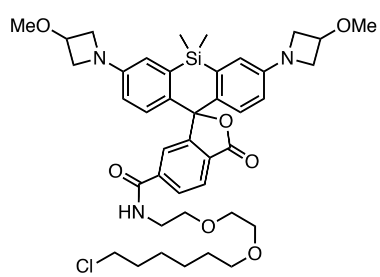


(20): Synthesized following procedure A from 6-*tert*-butoxycarbonylsilafluorescein ditriflate **4** and 3-(trifluoromethyl)azetidine hydrochloride. Purification by silica gel chromatography (0–20% EtOAc/hexane, linear gradient) afforded **20** (54%) as a light blue solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.12 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.82 (s, 1H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.68 (d, *J* = 2.6 Hz, 2H), 6.33 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.08 (t, *J* = 8.1 Hz, 4H), 3.98 (dt, *J* = 7.8, 5.6 Hz, 4H), 3.39 (qt, *J* = 8.5, 5.8 Hz, 2H), 1.55 (s, 9H), 0.66 (s, 3H), 0.59 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.2 (C), 164.4 (C), 155.0 (C), 149.5 (C), 137.4 (C), 136.4 (C), 133.6 (C), 130.1 (CH), 129.0 (C), 127.8 (CH), 125.8 (CH), 125.1 (CH), 125.0 (C), 115.8 (CH), 112.8 (CH), 91.3 (C), 82.5 (C), 51.33 (CH_2), 51.30 (CH_2), 33.2 (q, $^{3}J_{CF}$ = 32.1 Hz, C), 28.2 (CH_3), 0.2 (CH_3), -0.7 (CH_3); HRMS (ESI) calcd for $C_{35}H_{35}N_2O_4SiF_6$ $[M+H]^+$ 689.2270, found 689.2282.



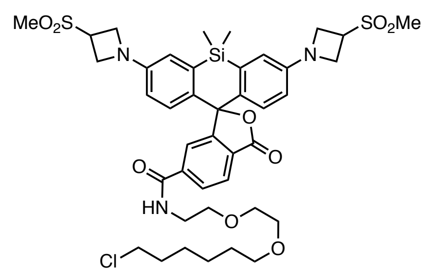
(21): Synthesized following procedure A from 6-*tert*-butoxycarbonylsilafluorescein ditriflate **4** and 3,3-difluoroazetidine hydrochloride. Purification by silica gel chromatography (0–30% EtOAc/hexane, linear gradient), followed by purification by silica gel chromatography (0–20% EtOAc/hexanes) afforded **21** (71%) as an off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.98 (dd, *J* = 8.1, 0.8 Hz, 1H), 7.82 (t, *J* = 1.0 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.73 (d, *J* = 2.7 Hz, 2H), 6.38 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.24 (t, *J* = 11.7 Hz, 8H), 1.55 (s, 9H), 0.68 (s, 3H), 0.61 (s, 3H); ^{19}F NMR ($CDCl_3$, 376 MHz) = -99.3 (p, $^{3}J_{HF}$ = 11.9 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.0 (C), 164.3 (C), 155.0 (C), 148.7 (t, $^{4}J_{HF}$ = 2.8 Hz, C), 137.5 (C), 136.5 (C), 134.3 (C), 130.2 (CH), 128.9 (C), 127.9 (CH), 125.9 (CH), 125.0 (C), 116.8 (CH), 115.9 (t, $^{1}J_{CF}$ = 276 Hz, CF_2), 113.9 (CH), 91.0 (C), 82.5 (C), 63.4 (t, $^{2}J_{HF}$ = 26.0 Hz, CH_2), 28.2 (CH_3), 0.2 (CH_3), -0.7 (CH_3); HRMS (ESI) calcd for $C_{33}H_{33}N_2O_4SiF_4$ $[M+H]^+$ 625.2146, found 625.2145.

Procedure B: Synthesis of HaloTag ligands. The following procedure for **6** is representative. **17** (36 mg, 59 μmol) was taken up in CH_2Cl_2 (2 mL) and trifluoroacetic acid (0.25 mL) was added. The reaction was stirred at room temperature overnight. Toluene (3 mL) was added, the reaction mixture was concentrated to dryness and then azeotroped with MeOH three times. The residue was combined with HaloTag(O_2)amine (TFA salt, 30 mg, 89 μmol, 1.5 eq), HATU (34 mg, 89 μmol, 1.5 eq) in DMF (1.5 mL). DIEA (52 μL, 295 μmol, 5.0 eq) was added and the mixture was stirred at room temperature for 4 h. It was subsequently evaporated to dryness and purified as described.

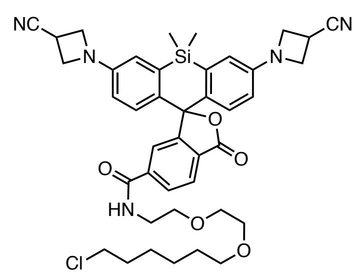


(6; JF_{639}-HaloTag ligand): Purification by silica gel chromatography (30–100% EtOAc/hexanes, linear gradient) provided **6** (60%) as a light-blue solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (dd, *J* = 8.0, 0.7 Hz, 1H), 7.91 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.68 (t, *J* = 1.0 Hz, 1H), 6.81 (br s, 1H), 6.76 (d, *J* = 8.6 Hz, 2H), 6.68 (d, *J* = 2.7 Hz, 2H), 6.29 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.37 – 4.29 (m, 2H), 4.13 – 4.07 (m, 4H), 3.76 – 3.70 (m, 4H), 3.66 – 3.60 (m, 6H), 3.56 – 3.52 (m, 2H), 3.50 (t, *J* = 6.7 Hz, 2H), 3.39 (t, *J* = 6.7 Hz, 2H), 3.32 (s, 6H), 1.78 – 1.69 (m, 2H), 1.51 (p, *J* = 6.9 Hz, 2H), 1.44 – 1.35 (m, 2H), 1.34 – 1.23 (m, 2H), 0.64 (s, 3H), 0.57 (s, 3H); Analytical HPLC: t_R = 13.0 min, 99% purity (10–95% MeCN/H_2O, linear gradient, with constant 0.1% v/v

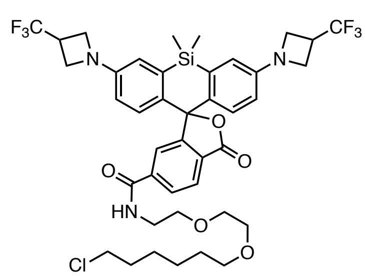
TFA additive, 20 min run, 1 mL/min flow, detection at 254 nm); HRMS (ESI) calculated for $C_{41}H_{53}ClN_3O_7Si$ $[M+H]^+$ 762.3341, found 762.3352.



(7; JF$_{630}$-HaloTag ligand): Synthesized following procedure B from **18**. Purification by silica gel chromatography (0–4% MeOH/CH_2Cl_2, linear gradient), followed by purification by silica gel chromatography (50–100% EtOAc/hexanes, linear gradient) afforded **7** (65%) as a light blue solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ.97 (d, *J* = 7.9 Hz, 1H), 7.89 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.68 (t, *J* = 1.0 Hz, 1H), 6.91 – 6.87 (m, 1H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.70 (d, *J* = 2.7 Hz, 2H), 6.32 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.28 – 4.17 (m, 8H), 4.13 – 4.03 (m, 2H), 3.67 – 3.59 (m, 6H), 3.57 – 3.54 (m, 2H), 3.50 (t, *J* = 6.7 Hz, 2H), 3.40 (t, *J* = 6.7 Hz, 2H), 2.96 (s, 6H), 1.78 – 1.67 (m, 2H), 1.51 (p, *J* = 6.8 Hz, 2H), 1.44 – 1.35 (m, 2H), 1.35 – 1.26 (m, 2H), 0.65 (s, 3H), 0.57 (s, 3H); Analytical HPLC: t_R = 13.0 min, 98% purity (10–95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive, 20 min run, 1 mL/min flow, detection at 254 nm); HRMS (ESI) calculated for $C_{41}H_{53}ClN_3O_9S_2Si$ $[M+H]^+$ 858.2681, found 858.2690.

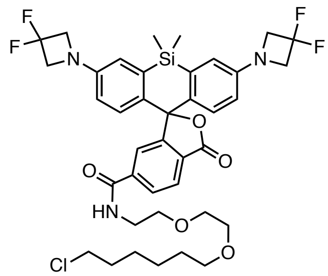


(8; JF$_{629}$-HaloTag ligand): Synthesized following procedure B from **19**. Purification by silica gel chromatography (20–100% EtOAc/hexanes, linear gradient) afforded **8** (73%) as a light blue solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (dd, *J* = 8.0, 0.7 Hz, 1H), 7.88 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.70 (t, *J* = 1.0 Hz, 1H), 6.89 – 6.82 (m, 3H), 6.67 (d, *J* = 2.6 Hz, 2H), 6.30 (dd, *J* = 8.7, 2.7 Hz, 2H), 4.20 (dd, *J* = 8.5, 7.0 Hz, 4H), 4.09 (q, *J* = 6.7 Hz, 4H), 3.65 – 3.54 (m, 10H), 3.50 (t, *J* = 6.6 Hz, 2H), 3.41 (t, *J* = 6.7 Hz, 2H), 1.77 – 1.69 (m, 4H), 1.52 (p, *J* = 6.9 Hz, 2H), 1.44 – 1.36 (m, 2H), 1.35 – 1.28 (m, 2H), 0.66 (s, 3H), 0.58 (s, 3H); Analytical HPLC: t_R = 14.4 min, 97% purity (10–95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive, 20 min run, 1 mL/min flow, detection at 254 nm); HRMS (ESI) calculated for $C_{41}H_{47}ClN_5O_5Si$ $[M+H]^+$ 752.3035, found 752.3044.



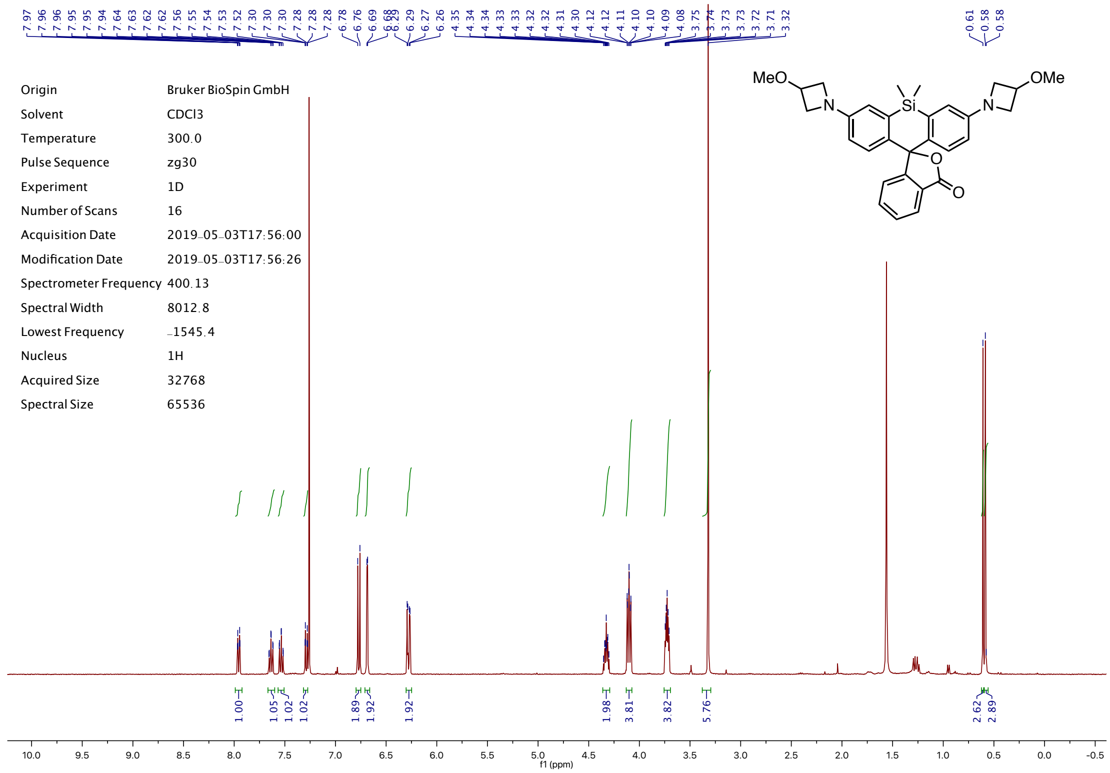
(9; JF$_{626}$-HaloTag ligand): Synthesized following procedure B from **20**. Purification by silica gel chromatography (30–100% EtOAc/hexanes, linear gradient) afforded **9** (83%) as a light blue solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ^{1}H 7.99 (d, *J* = 7.8 Hz, 1H), 7.89 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.73 – 7.68 (m, 1H), 6.84 –

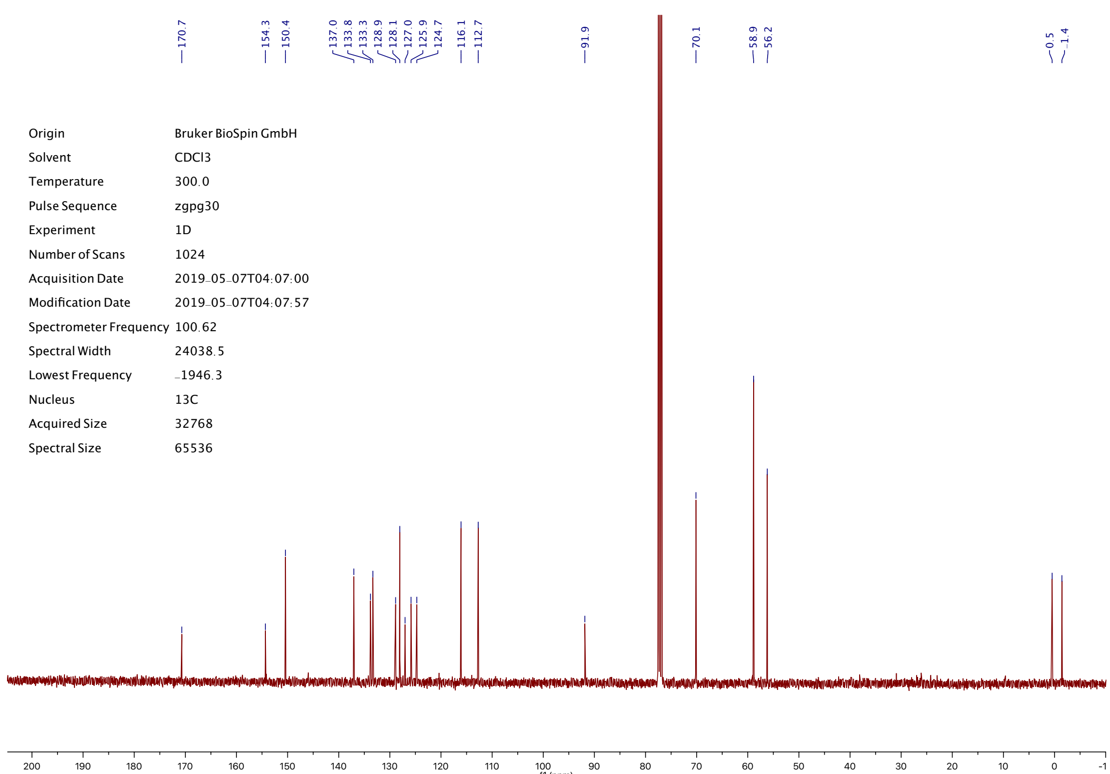
6.78 (m, 3H), 6.67 (d, J = 2.7 Hz, 2H), 6.29 (dd, J = 8.7, 2.7 Hz, 2H), 4.07 (t, J = 8.1 Hz, 4H), 4.01 – 3.92 (m, 4H), 3.68 – 3.60 (m, 6H), 3.58 – 3.54 (m, 2H), 3.50 (t, J = 6.6 Hz, 2H), 3.45 – 3.34 (m, 4H), 1.77 – 1.68 (m, 2H), 1.56 – 1.46 (m, 2H), 1.44 – 1.35 (m, 2H), 1.34 – 1.26 (m, 2H), 0.65 (s, 3H), 0.58 (s, 3H); ^{19}F NMR ($CDCl_3$, 376 MHz) = -73.5 (d, $^3J_{HF}$ = 8.7 Hz); Analytical HPLC: t_R = 16.4 min, 98% purity (10–95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive, 20 min run, 1 mL/min flow, detection at 254 nm); HRMS (ESI) calculated for $C_{41}H_{47}ClF_6N_3O_5Si$ [M+H]$^+$ 838.2878, found 838.2891.

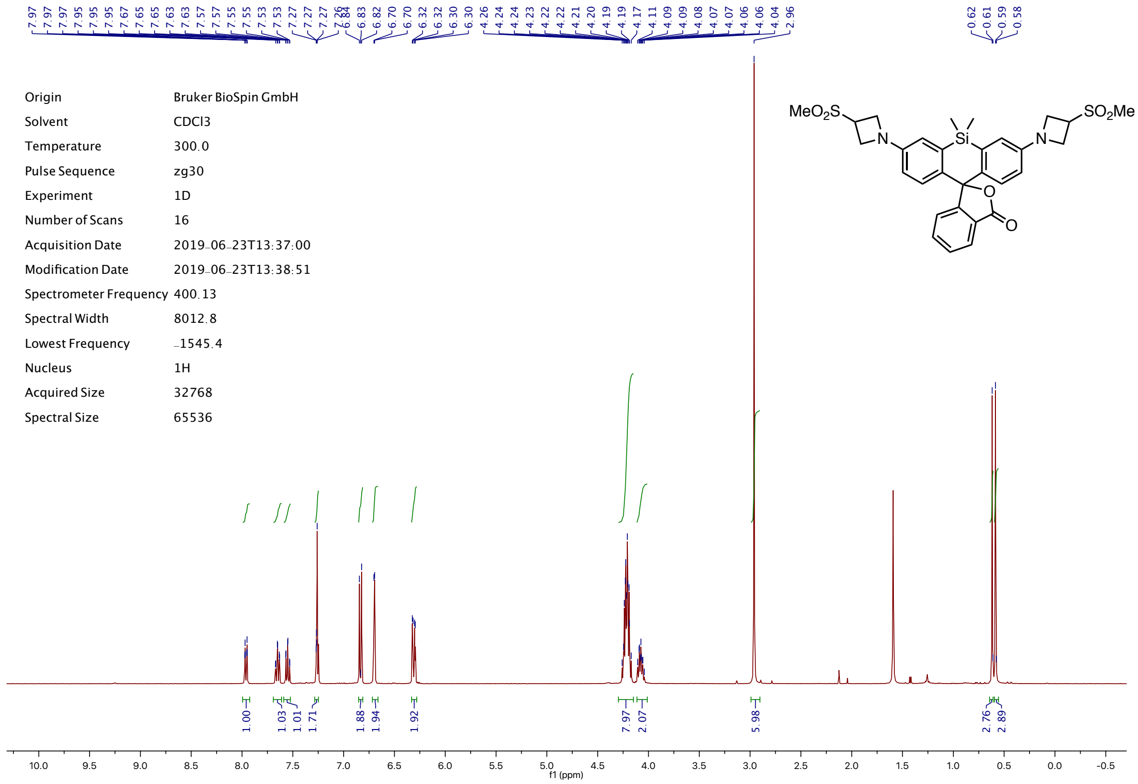


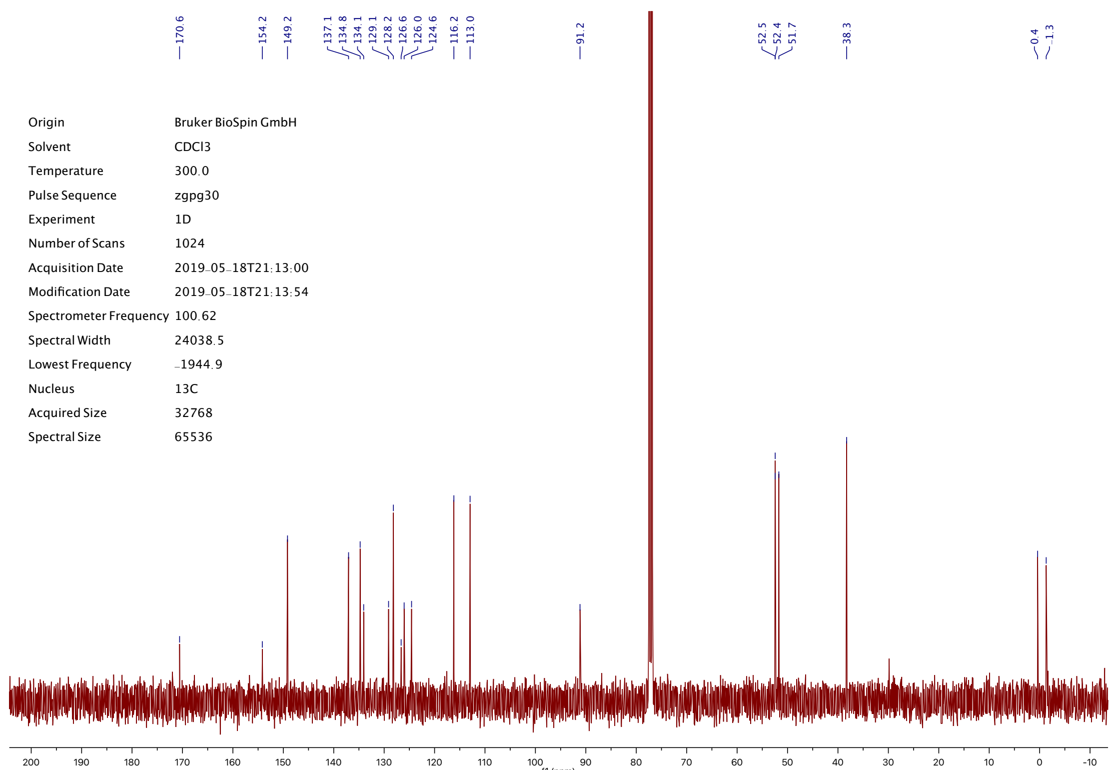
(10; JF$_{614}$-HaloTag ligand): Synthesized following procedure B from **21**. Purification by silica gel chromatography (0–3% MeOH/CH_2Cl_2, linear gradient) afforded **10** (75%) as an off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (dd, J = 7.9, 0.7 Hz, 1H), 7.88 (dd, J = 8.0, 1.4 Hz, 1H), 7.70 (t, J = 1.0 Hz, 1H), 6.87 (d, J = 8.7 Hz, 2H), 6.77 – 6.70 (m, 3H), 6.36 (dd, J = 8.7, 2.7 Hz, 2H), 4.24 (t, J = 11.7 Hz, 8H), 3.67 – 3.59 (m, 6H), 3.58 – 3.53 (m, 2H), 3.50 (t, J = 6.6 Hz, 2H), 3.41 (t, J = 6.7 Hz, 2H), 1.78 – 1.69 (m, 2H), 1.57 – 1.49 (m, 2H), 1.44 – 1.36 (m, 2H), 1.35 – 1.28 (m, 2H), 0.67 (s, 3H), 0.60 (s, 3H); ^{19}F NMR ($CDCl_3$, 376 MHz) = - 99.9 (p, $^3J_{HF}$ = 11.6 Hz); Analytical HPLC: t_R = 16.3 min, 95% purity (10–95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive, 20 min run, 1 mL/min flow, detection at 254 nm); HRMS (ESI) calculated for $C_{39}H_{45}ClF_4N_3O_5Si$ [M+H]$^+$ 774.2753, found 774.2759.

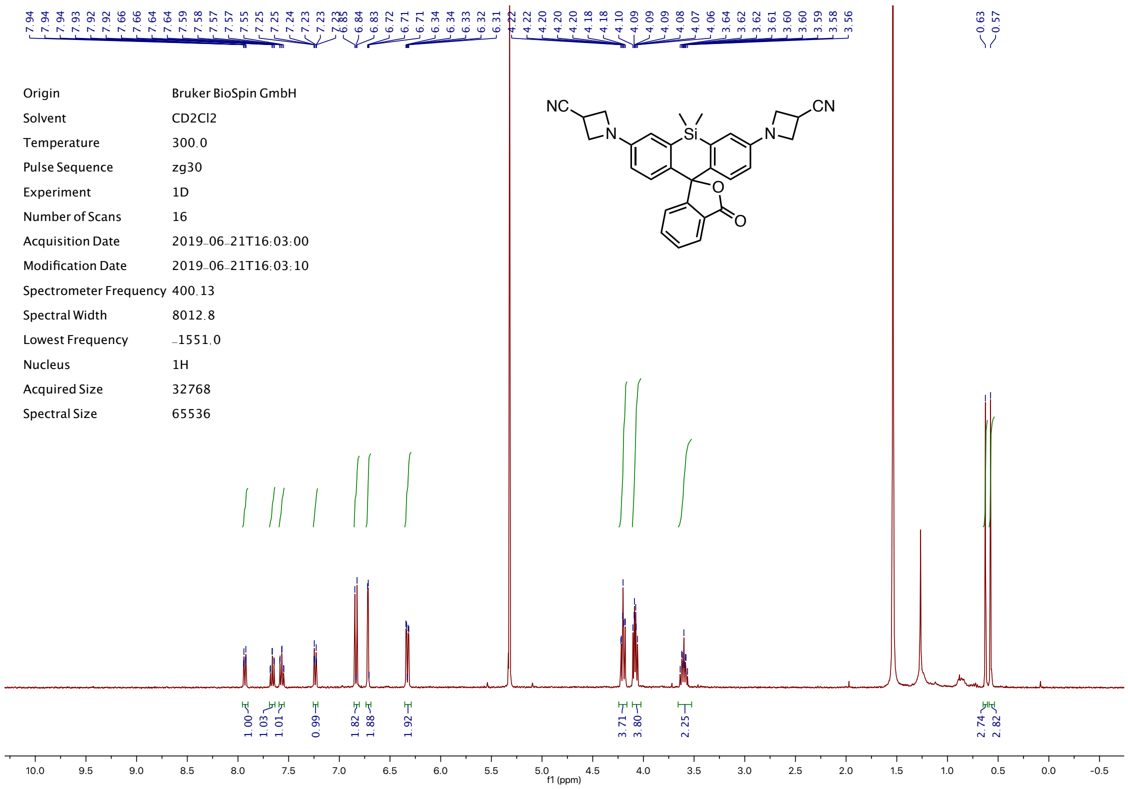
NMR SPECTRA AND HPLC TRACES



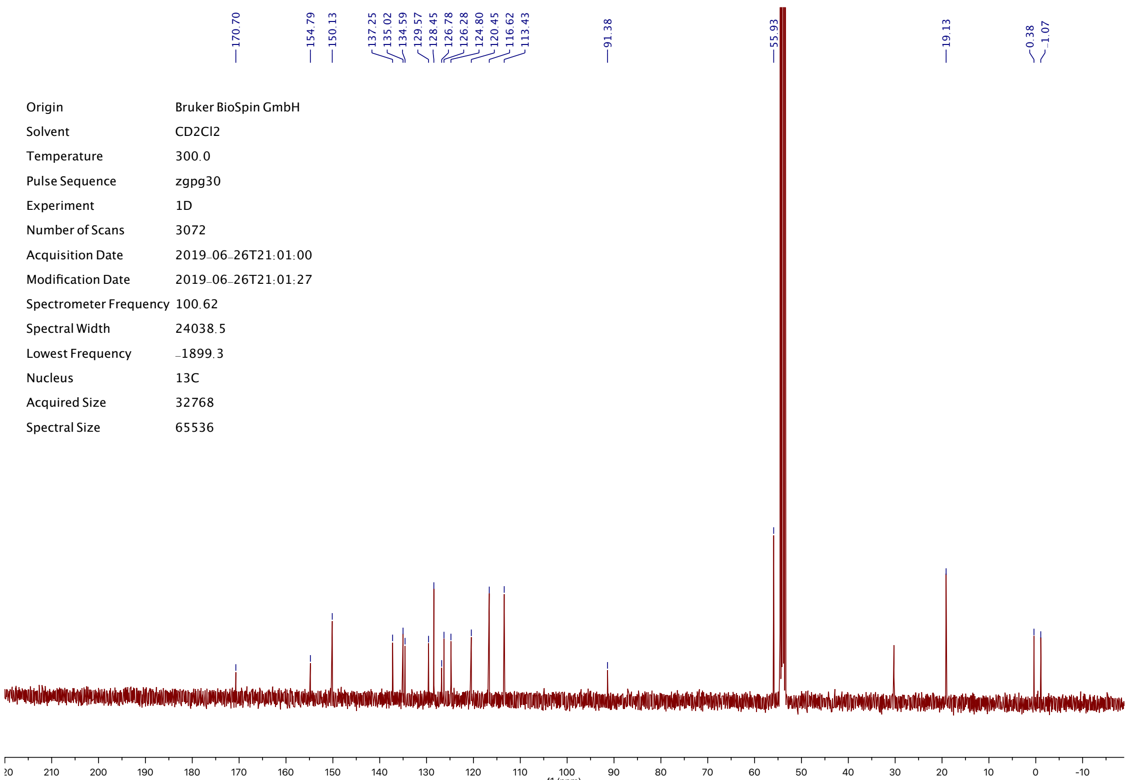



7.97 7.97 7.97 7.95 7.95 7.95 7.67 7.65 7.65 7.63 7.63 7.57 7.57 7.55 7.55 7.53 7.27 7.27 7.27 7.26 6.84 6.83 6.82 6.70 6.70 6.32 6.32 6.30 6.30 4.26 4.24 4.24 4.23 4.22 4.22 4.21 4.20 4.19 4.19 4.17 4.11 4.09 4.09 4.08 4.07 4.07 4.06 4.06 4.04 2.96
0.62 0.61 0.59 0.58
Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2019-06-23T13:37:00
Modification Date 2019-06-23T13:38:51
Spectrometer Frequency 400.13
Spectral Width 8012.8
Lowest Frequency -1545.4
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
MeO2S
SO2Me
N
Si
O
O
1.00 1.03 1.01 1.71 1.88 1.94 1.92 7.97 2.07 5.98 2.76 2.89
f1 (ppm)

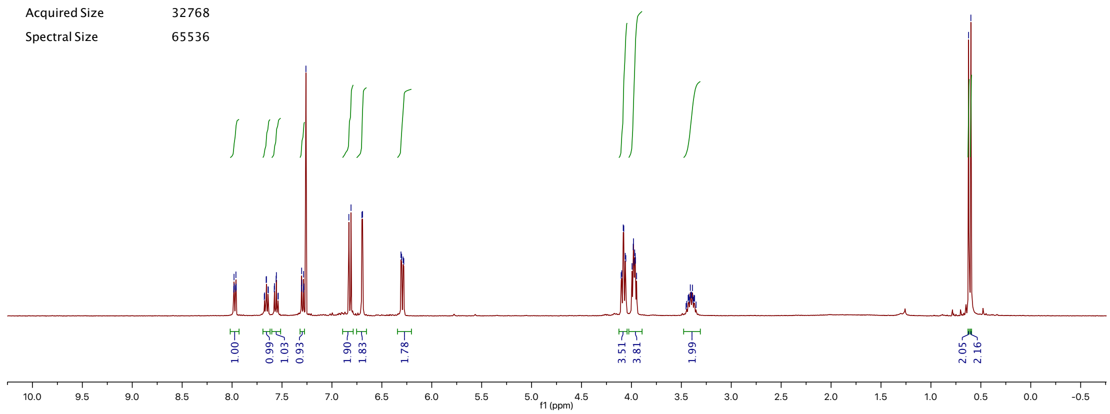

170.6 154.2 149.2 137.1 134.8 134.1 129.1 128.2 126.6 126.0 124.6 116.2 113.0 91.2 52.5 52.4 51.7 38.3 -0.4 -1.3
Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zgpg30
Experiment 1D
Number of Scans 1024
Acquisition Date 2019-05-18T21:13:00
Modification Date 2019-05-18T21:13:54
Spectrometer Frequency 100.62
Spectral Width 24038.5
Lowest Frequency -1944.9
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
f1 (ppm)



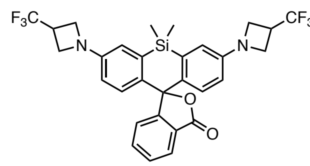
Origin	Bruker BioSpin GmbH
Solvent	CD2Cl2
Temperature	300.0
Pulse Sequence	zg30
Experiment	1D
Number of Scans	16
Acquisition Date	2019-06-21T16:03:00
Modification Date	2019-06-21T16:03:10
Spectrometer Frequency	400.13
Spectral Width	8012.8
Lowest Frequency	-1551.0
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

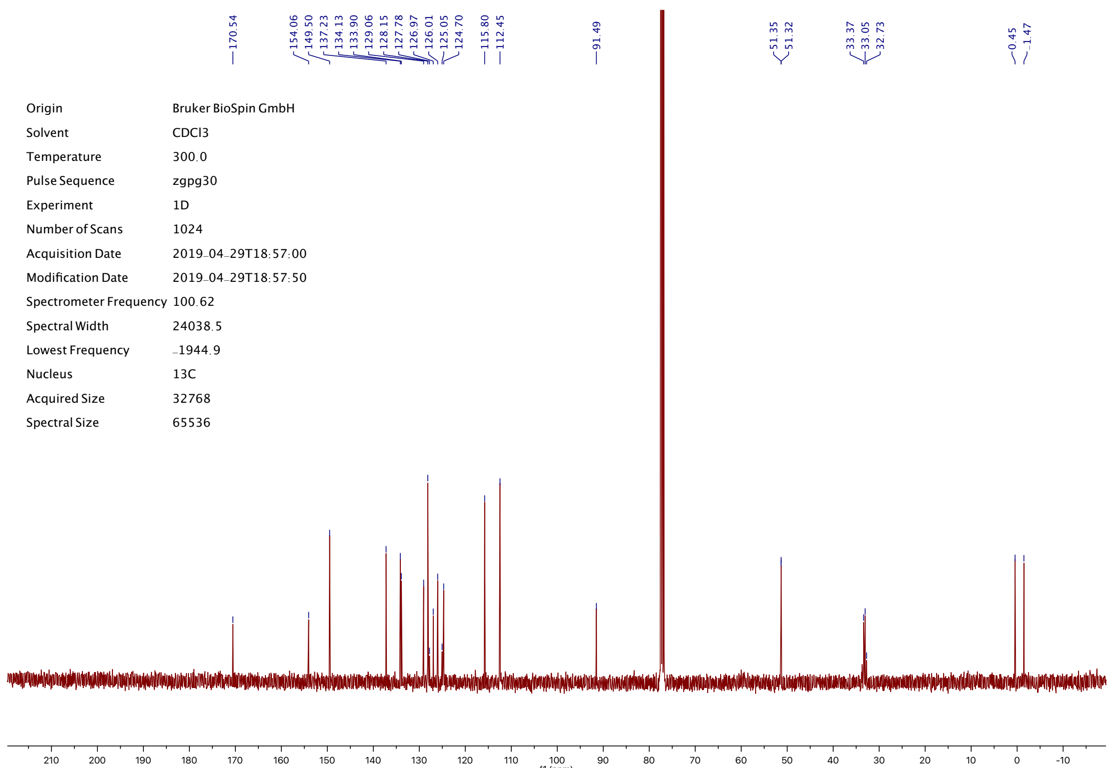


Origin	Bruker BioSpin GmbH
Solvent	CD2Cl2
Temperature	300.0
Pulse Sequence	zgpg30
Experiment	1D
Number of Scans	3072
Acquisition Date	2019-06-26T21:01:00
Modification Date	2019-06-26T21:01:27
Spectrometer Frequency	100.62
Spectral Width	24038.5
Lowest Frequency	-1899.3
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

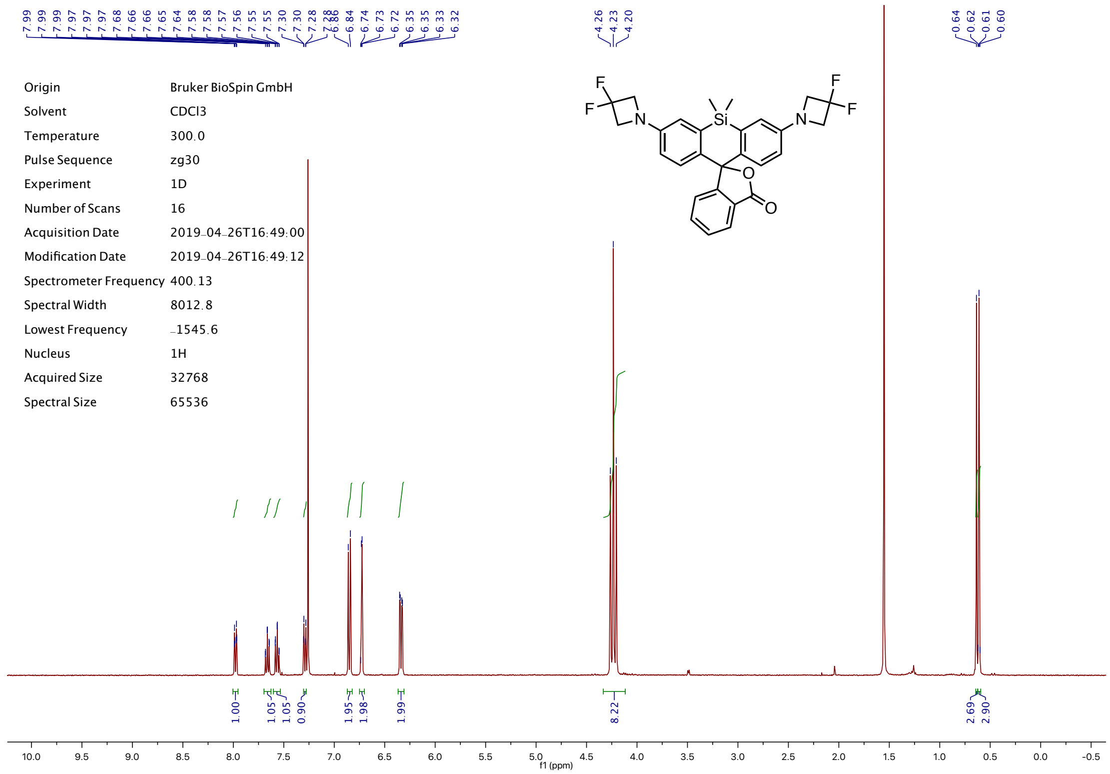


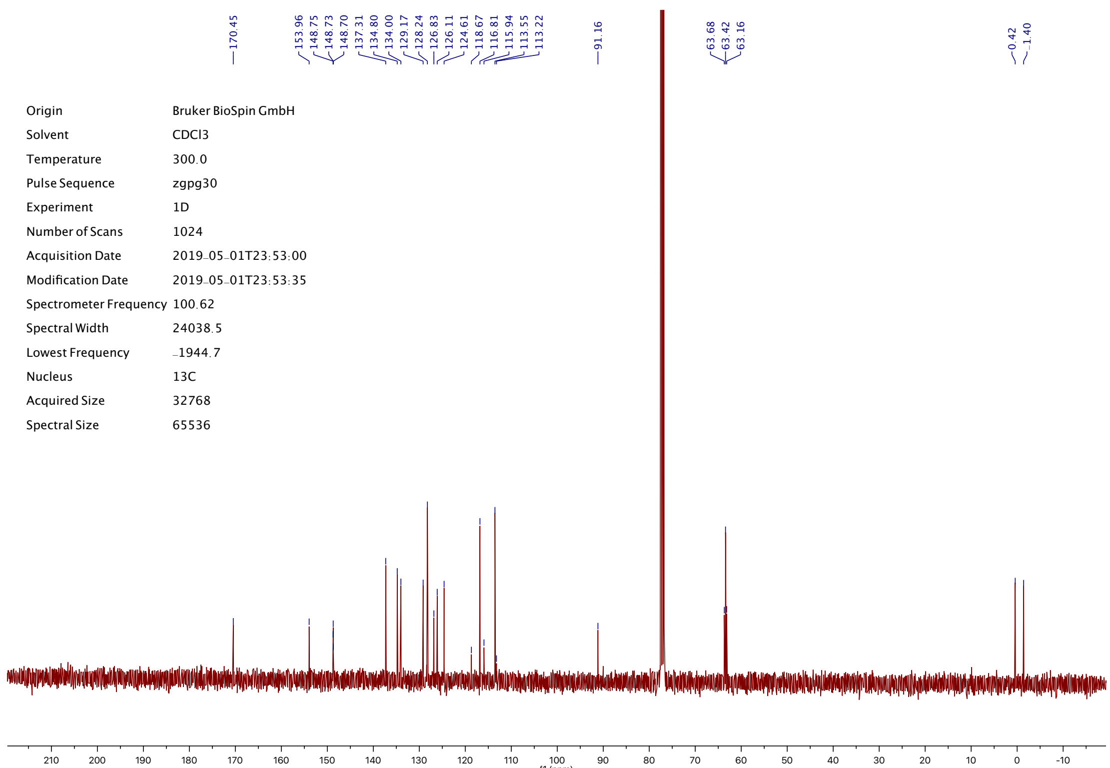
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zg30
Experiment	1D
Number of Scans	16
Acquisition Date	2019-06-18T09:53:00
Modification Date	2019-06-18T09:53:19
Spectrometer Frequency	400.13
Spectral Width	8012.8
Lowest Frequency	-1545.4
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

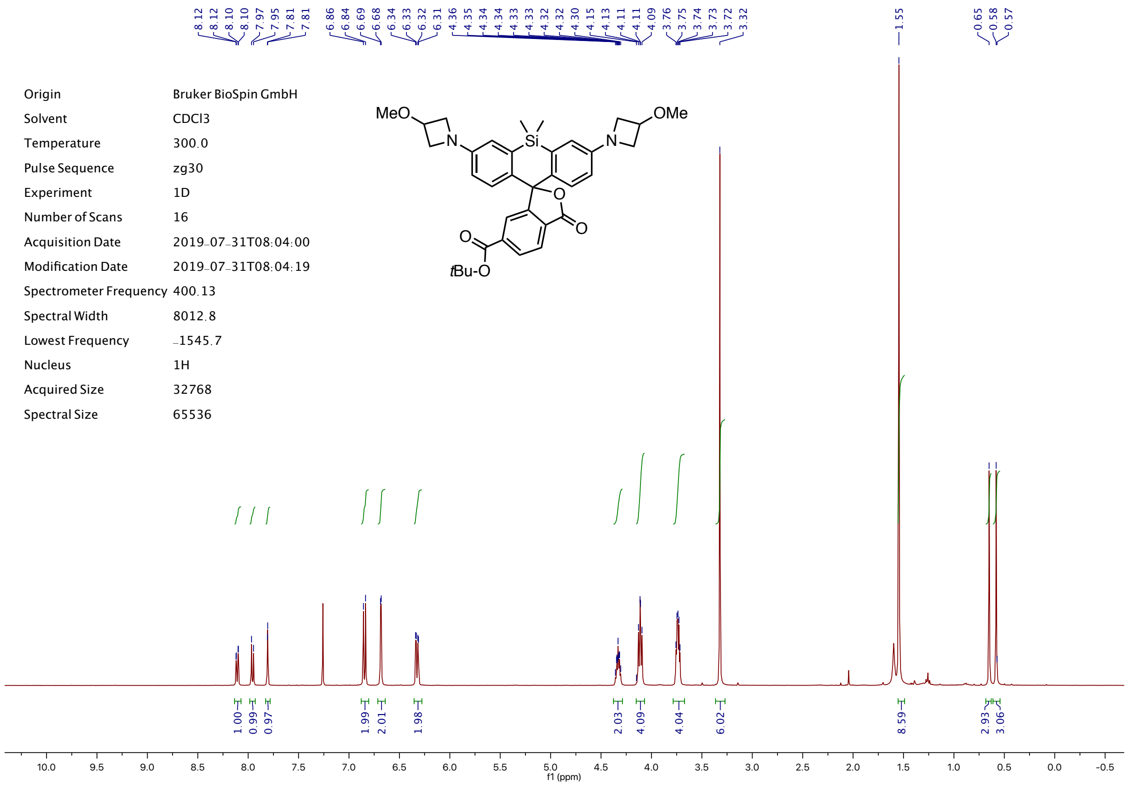




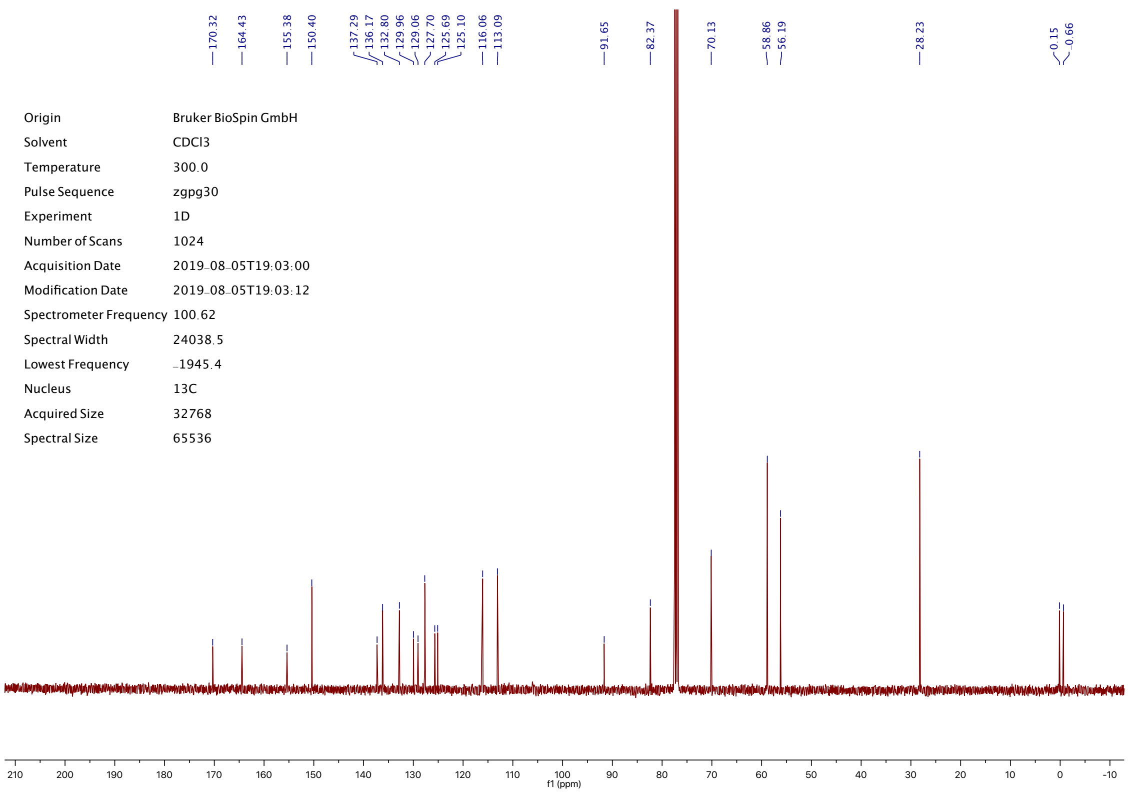
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zgpg30
Experiment	1D
Number of Scans	1024
Acquisition Date	2019-04-29T18:57:00
Modification Date	2019-04-29T18:57:50
Spectrometer Frequency	100.62
Spectral Width	24038.5
Lowest Frequency	-1944.9
Nucleus	13C
Acquired Size	32768
Spectral Size	65536


Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2019-04-26T16:49:00
Modification Date 2019-04-26T16:49:12
Spectrometer Frequency 400.13
Spectral Width 8012.8
Lowest Frequency -1545.6
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
f1 (ppm)

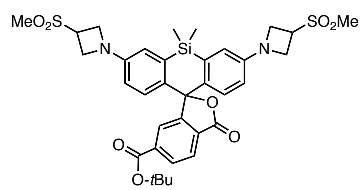

Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zgpg30
Experiment 1D
Number of Scans 1024
Acquisition Date 2019-05-01T23:53:00
Modification Date 2019-05-01T23:53:35
Spectrometer Frequency 100.62
Spectral Width 24038.5
Lowest Frequency -1944.7
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
f1 (ppm)



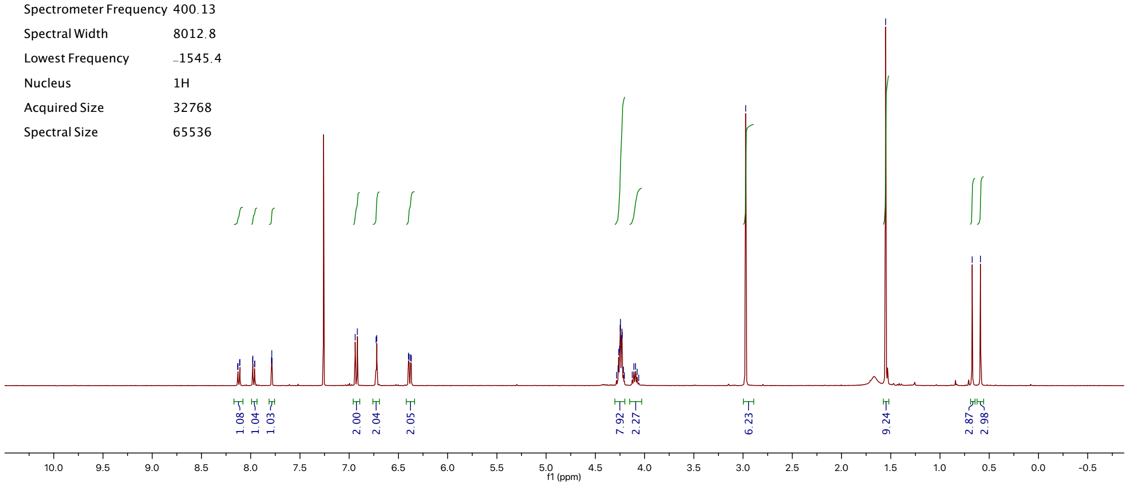
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zg30
Experiment	1D
Number of Scans	16
Acquisition Date	2019-07-31T08:04:00
Modification Date	2019-07-31T08:04:19
Spectrometer Frequency	400.13
Spectral Width	8012.8
Lowest Frequency	-1545.7
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



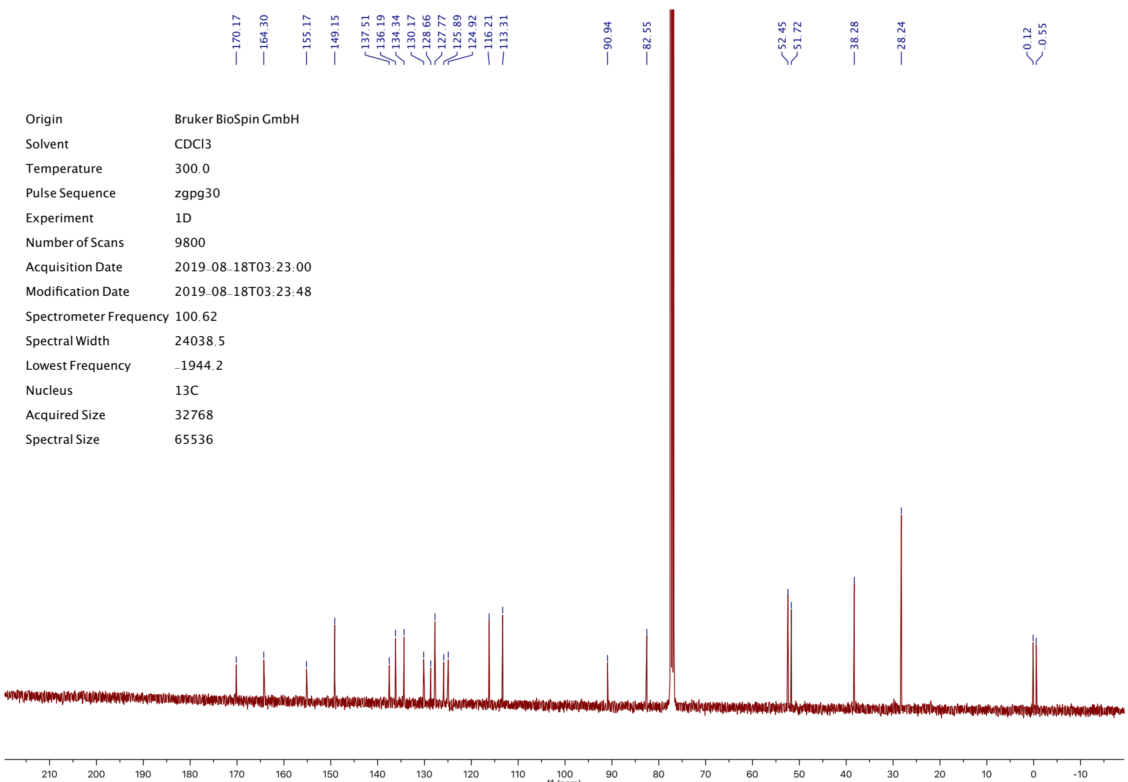
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zgpg30
Experiment	1D
Number of Scans	1024
Acquisition Date	2019-08-05T19:03:00
Modification Date	2019-08-05T19:03:12
Spectrometer Frequency	100.62
Spectral Width	24038.5
Lowest Frequency	-1945.4
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

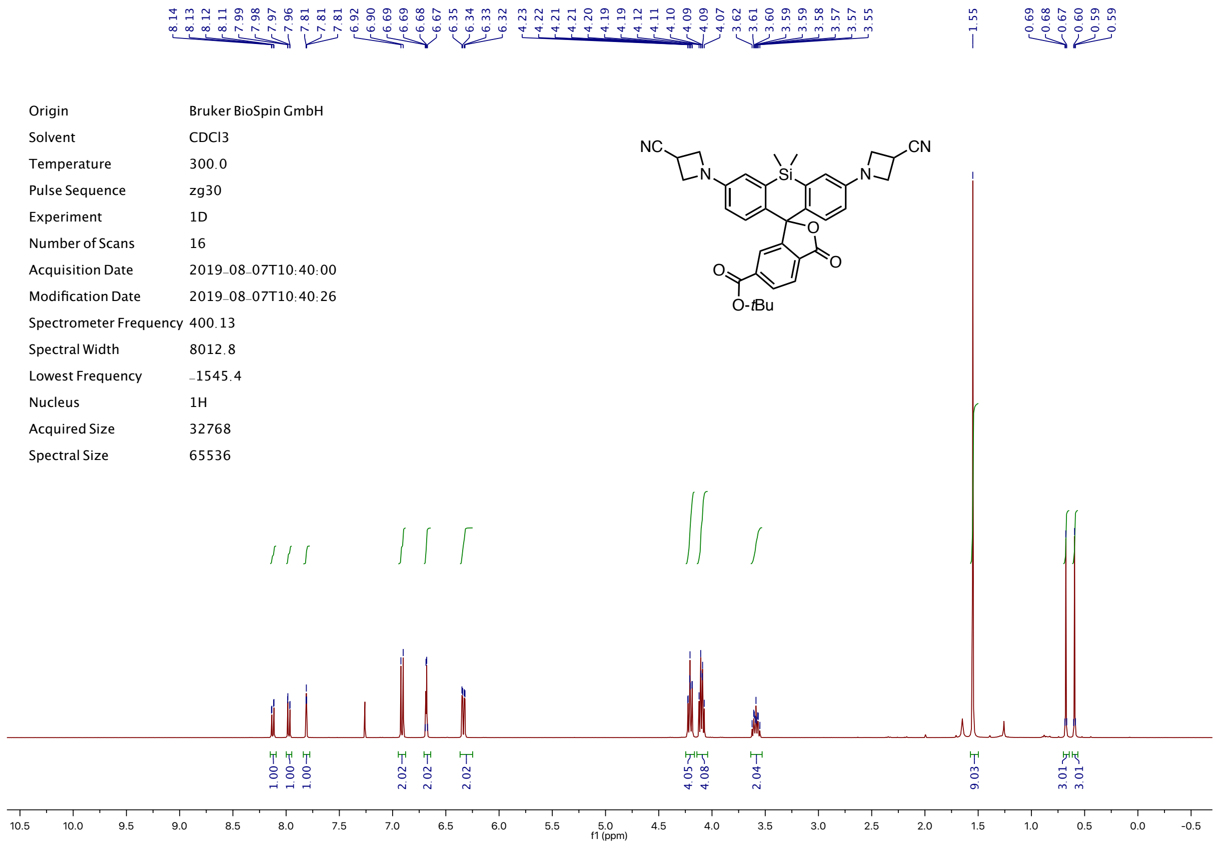


Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zg30
Experiment	1D
Number of Scans	16
Acquisition Date	2019-08-23T10:58:00
Modification Date	2019-08-23T10:58:39
Spectrometer Frequency	400.13
Spectral Width	8012.8
Lowest Frequency	-1545.4
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

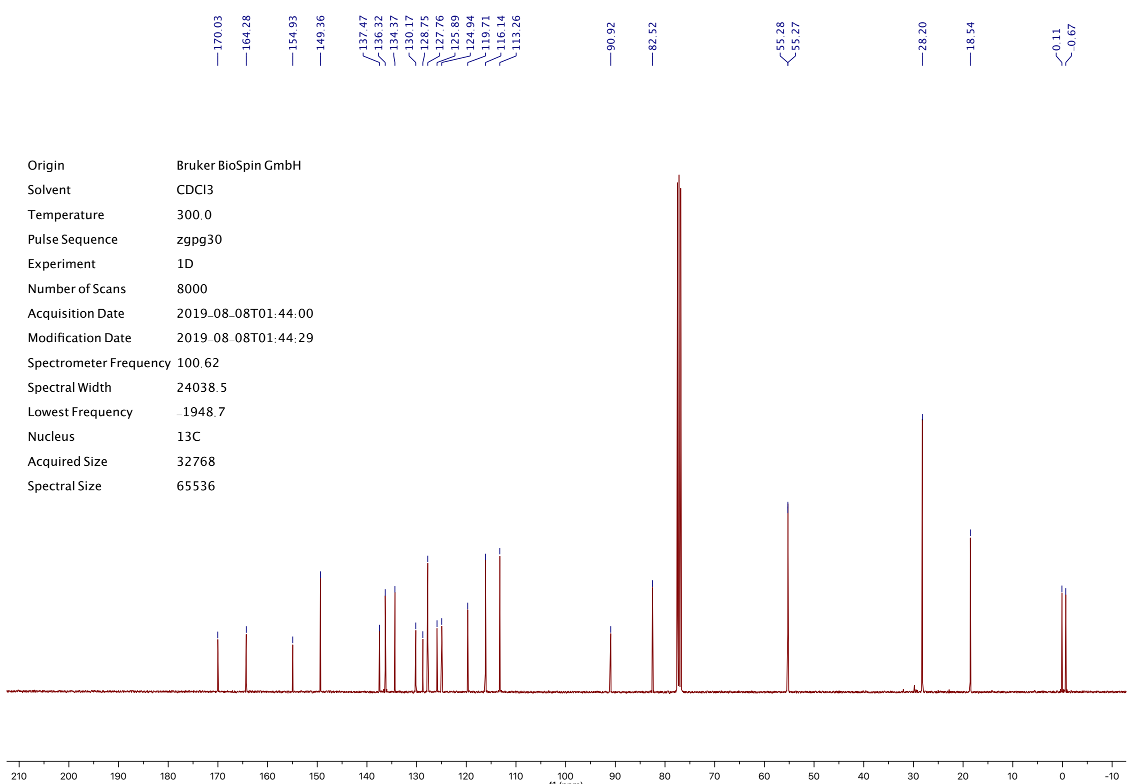


Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zgpg30
Experiment	1D
Number of Scans	9800
Acquisition Date	2019-08-18T03:23:00
Modification Date	2019-08-18T03:23:48
Spectrometer Frequency	100.62
Spectral Width	24038.5
Lowest Frequency	-1944.2
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

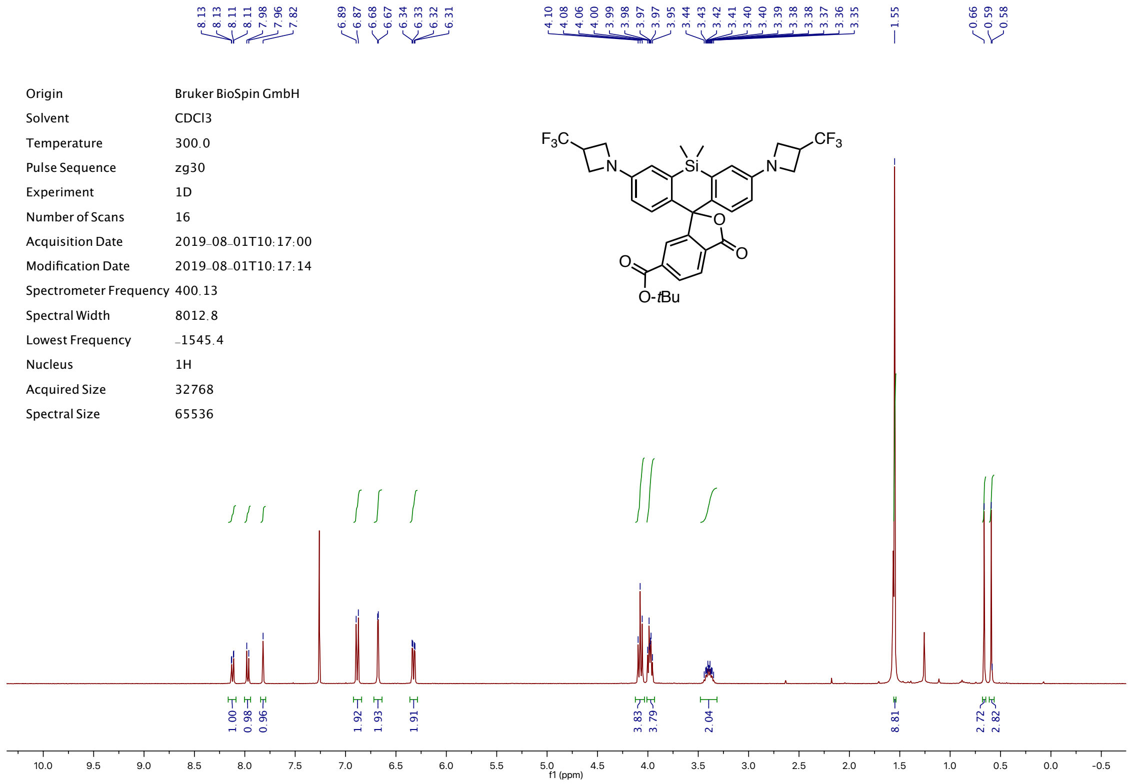


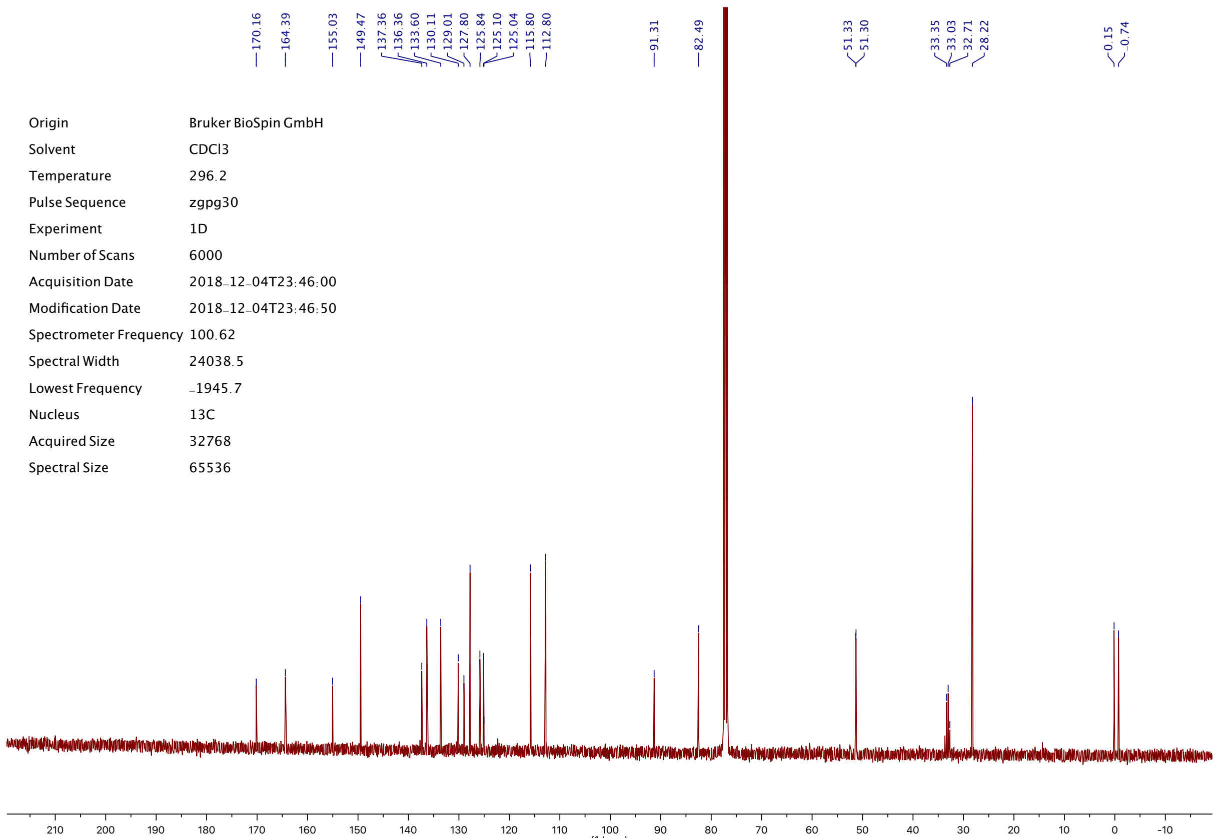


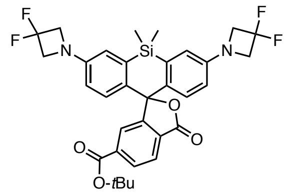
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zg30
Experiment	1D
Number of Scans	16
Acquisition Date	2019-08-07T10:40:00
Modification Date	2019-08-07T10:40:26
Spectrometer Frequency	400.13
Spectral Width	8012.8
Lowest Frequency	-1545.4
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

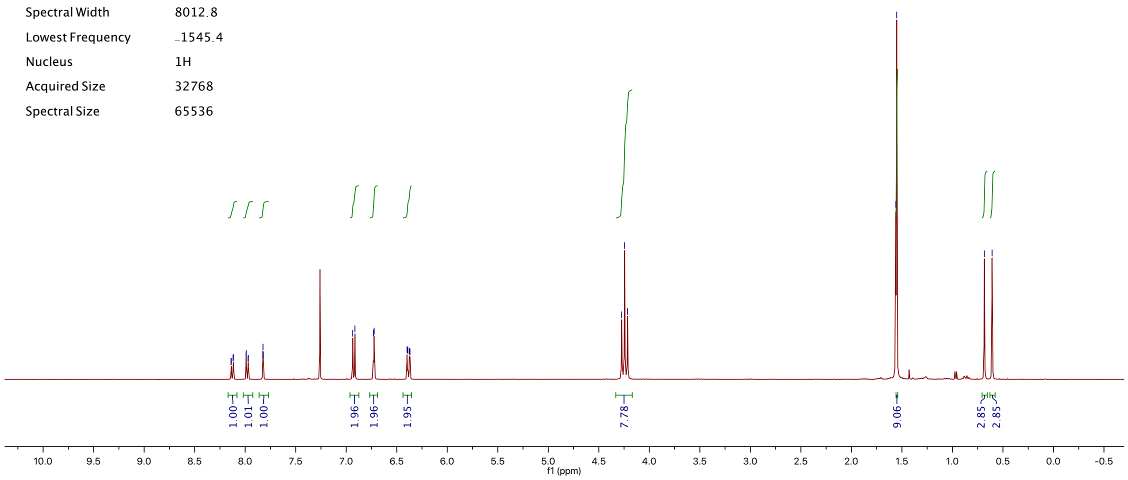


Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zgpg30
Experiment	1D
Number of Scans	8000
Acquisition Date	2019-08-08T01:44:00
Modification Date	2019-08-08T01:44:29
Spectrometer Frequency	100.62
Spectral Width	24038.5
Lowest Frequency	-1948.7
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

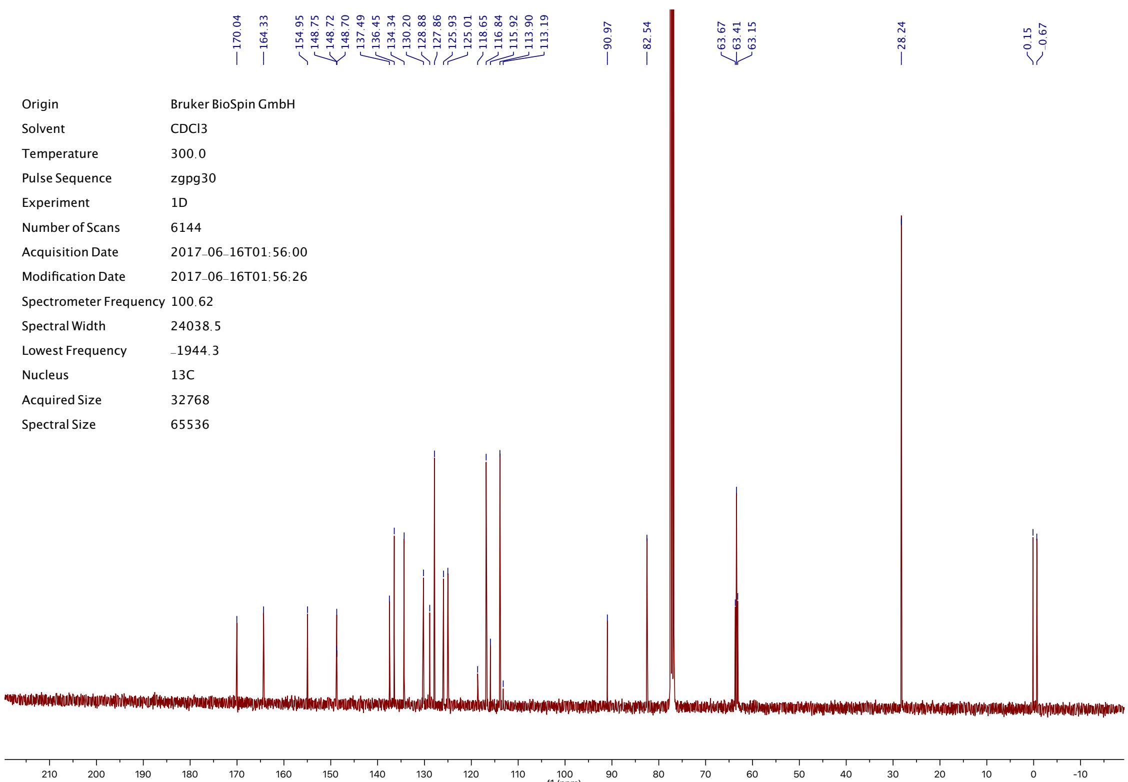

8.13 8.13 8.11 8.11 7.98 7.96 7.82
6.89 6.87 6.68 6.67 6.34 6.33 6.32 6.31
4.10 4.08 4.06 4.00 3.99 3.98 3.97 3.97 3.95 3.44 3.43 3.42 3.41 3.40 3.40 3.39 3.38 3.38 3.37 3.36 3.35
1.55
0.66 0.59 0.58
Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2019-08-01T10:17:00
Modification Date 2019-08-01T10:17:14
Spectrometer Frequency 400.13
Spectral Width 8012.8
Lowest Frequency -1545.4
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
F3C
N
Si
N
CF3
O
O
O
O-tBu
1.00 0.98 0.96 1.92 1.93 1.91 3.83 3.79 2.04 8.81 2.72 2.82
10.0 9.5 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)


170.16 164.39 155.03 149.47 137.36 136.36 133.60 130.11 129.01 127.80 125.84 125.10 125.04 115.80 112.80 91.31 82.49 51.33 51.30 33.35 33.03 32.71 28.22 0.15 -0.74
Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 296.2
Pulse Sequence zgpg30
Experiment 1D
Number of Scans 6000
Acquisition Date 2018-12-04T23:46:00
Modification Date 2018-12-04T23:46:50
Spectrometer Frequency 100.62
Spectral Width 24038.5
Lowest Frequency -1945.7
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

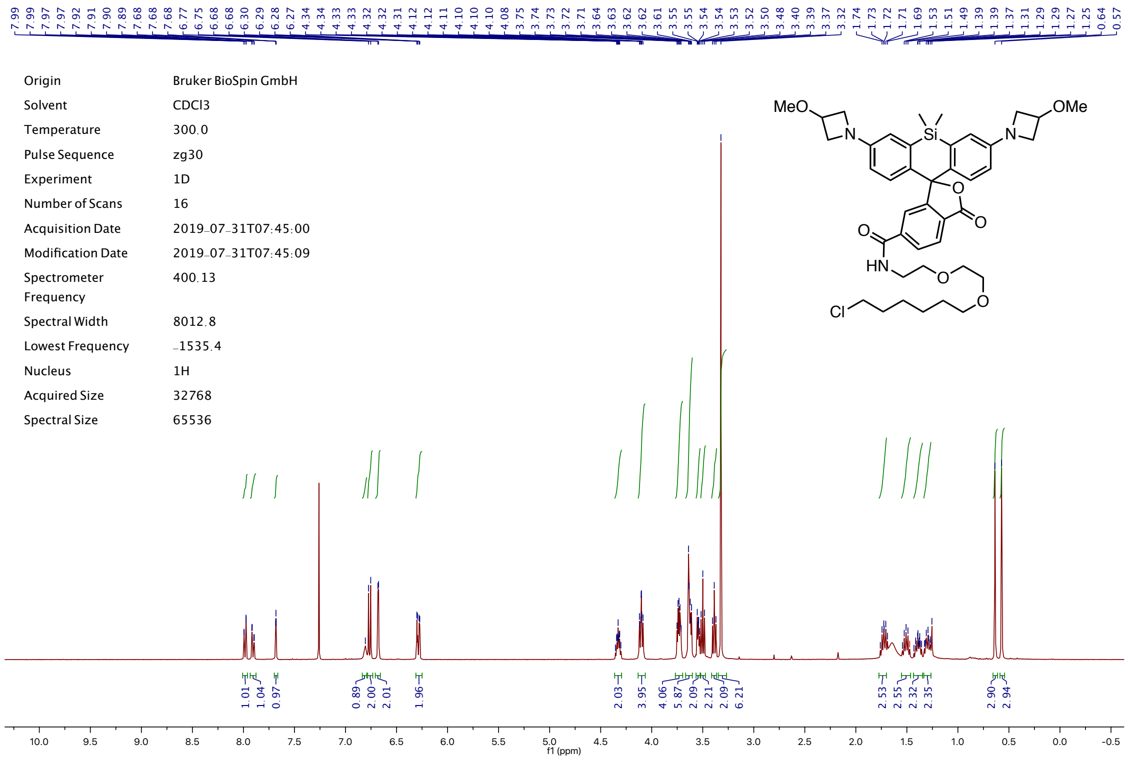


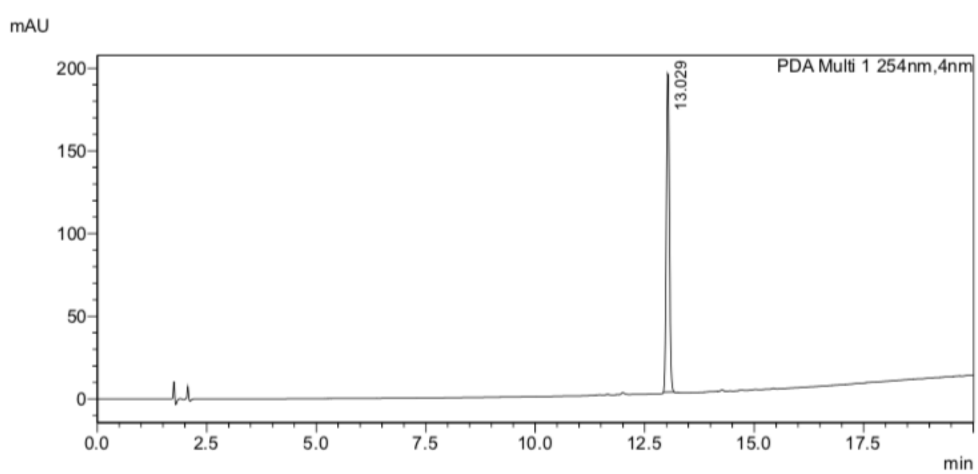


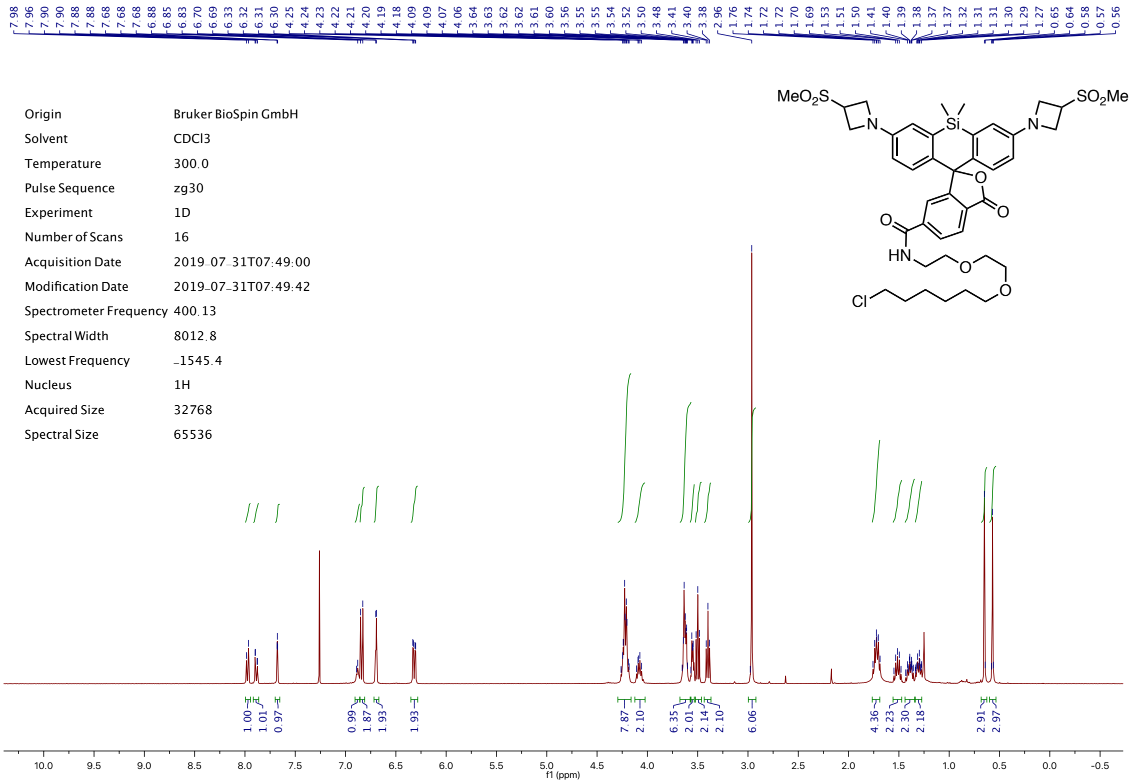
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zg30
Experiment	1D
Number of Scans	16
Acquisition Date	2019-08-07T09:53:00
Modification Date	2019-08-07T09:53:10
Spectrometer Frequency	400.13
Spectral Width	8012.8
Lowest Frequency	-1545.4
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

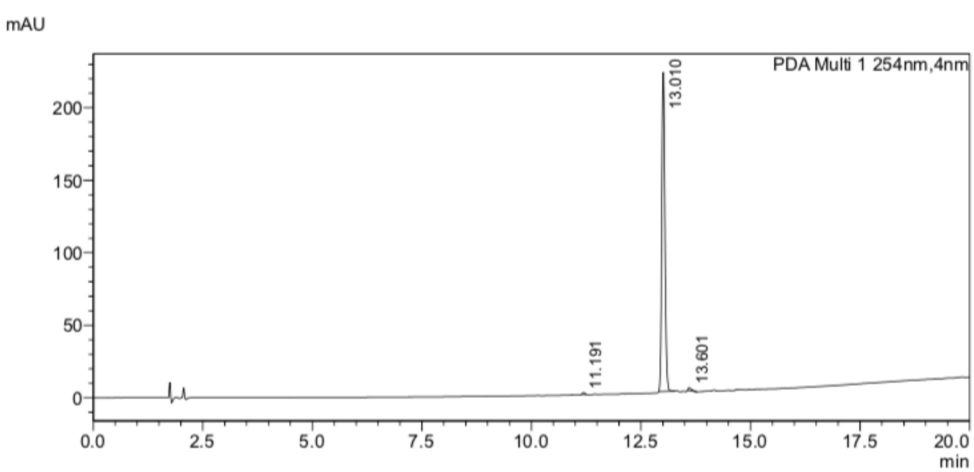


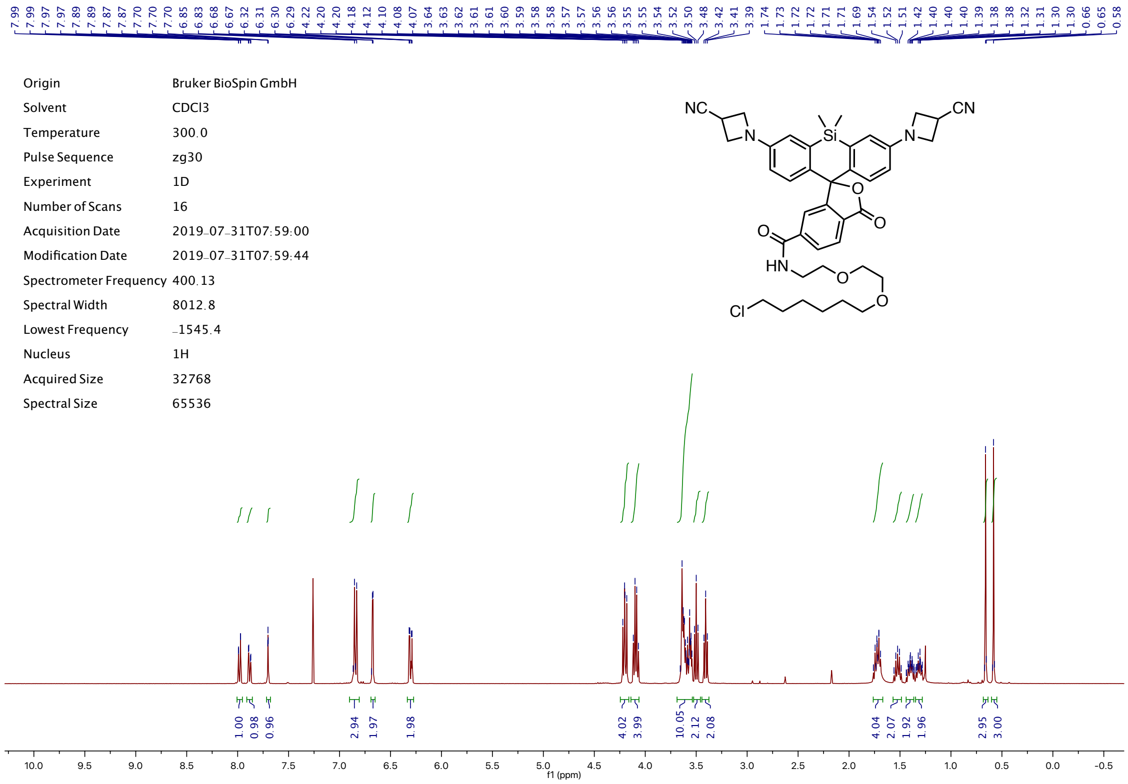
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Temperature	300.0
Pulse Sequence	zgpg30
Experiment	1D
Number of Scans	6144
Acquisition Date	2017-06-16T01:56:00
Modification Date	2017-06-16T01:56:26
Spectrometer Frequency	100.62
Spectral Width	24038.5
Lowest Frequency	-1944.3
Nucleus	13C
Acquired Size	32768
Spectral Size	65536


Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2019-07-31T07:45:00
Modification Date 2019-07-31T07:45:09
Spectrometer Frequency 400.13
Spectral Width 8012.8
Lowest Frequency -1535.4
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
MeO
OMe
Si
N
O
HN
Cl
f1 (ppm)

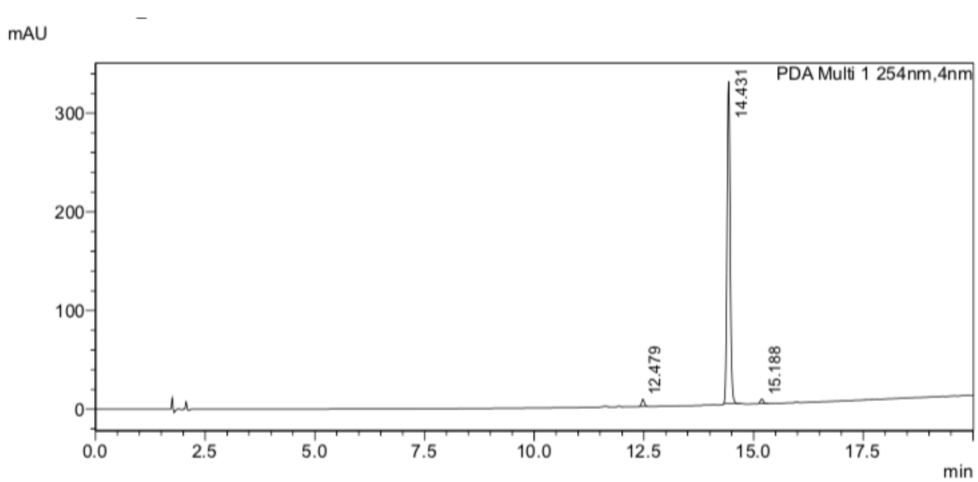

mAU
PDA Multi 1 254nm,4nm
13.029
min


Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2019-07-31T07:49:00
Modification Date 2019-07-31T07:49:42
Spectrometer Frequency 400.13
Spectral Width 8012.8
Lowest Frequency -1545.4
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
MeO2S
SO2Me
Si
N
O
HN
Cl
f1 (ppm)

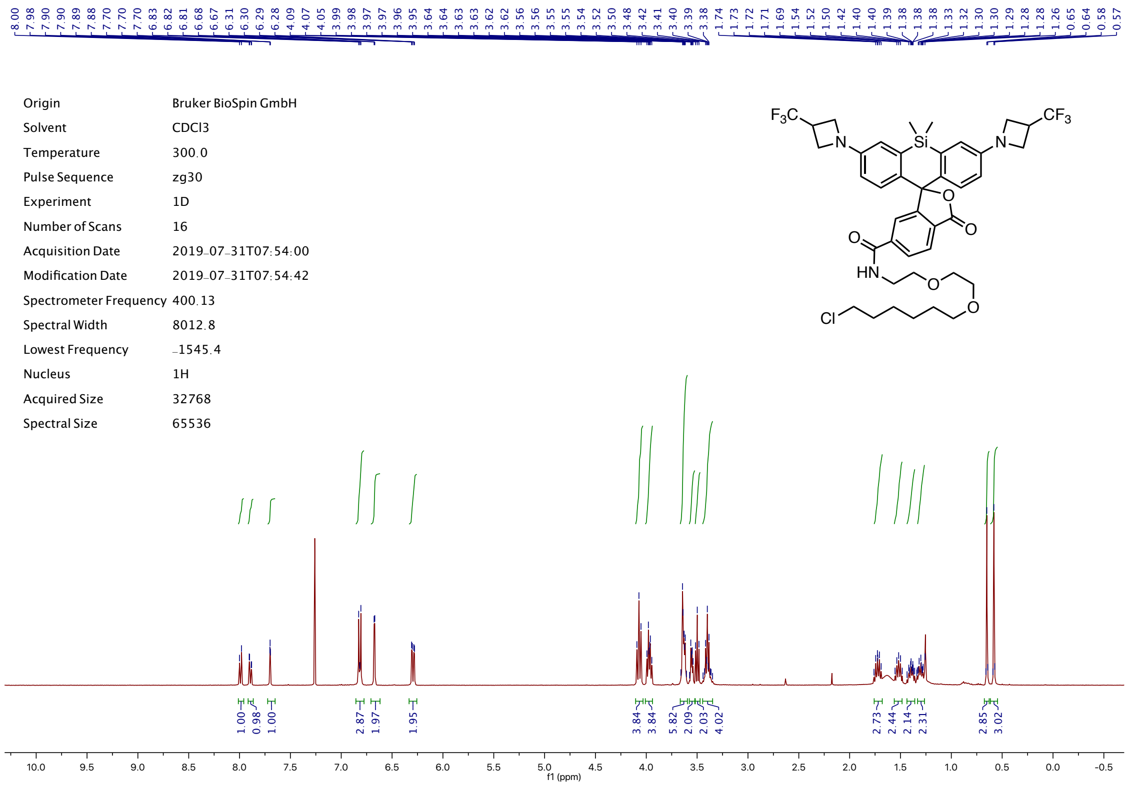

mAU
PDA Multi 1 254nm,4nm
13.010
11.191
13.601
min

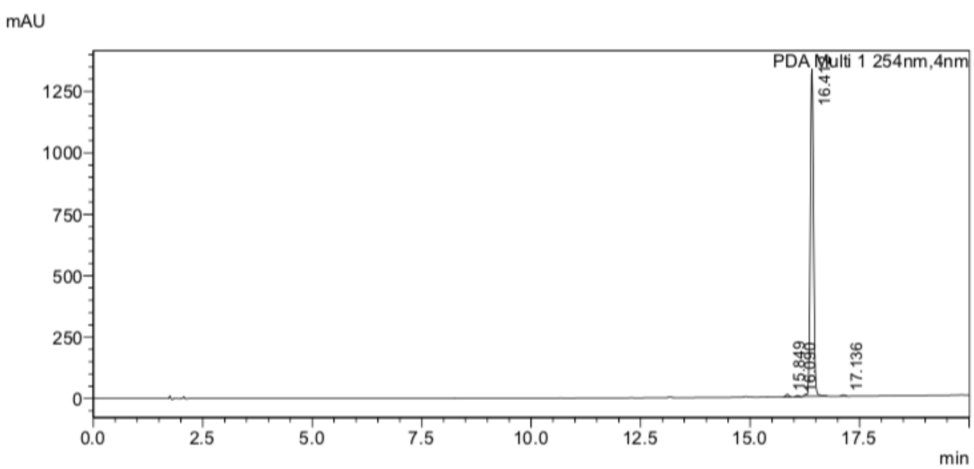


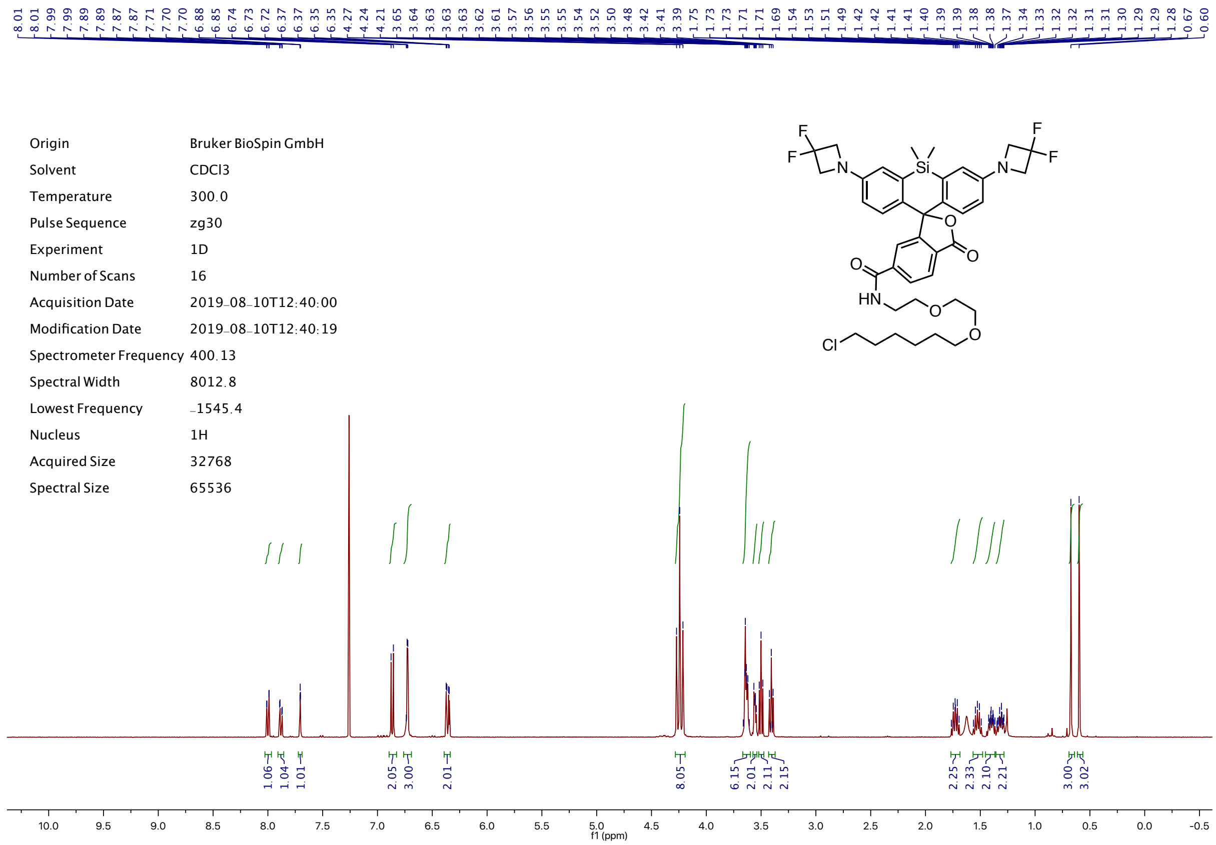
Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2019-07-31T07:59:00
Modification Date 2019-07-31T07:59:44
Spectrometer Frequency 400.13
Spectral Width 8012.8
Lowest Frequency -1545.4
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
NC
CN
N
Si
O
HN
Cl
1.00 0.98 0.96 2.94 1.97 1.98 4.02 3.99 10.05 2.12 2.08 4.04 2.07 1.92 1.96 2.95 3.00
f1 (ppm)

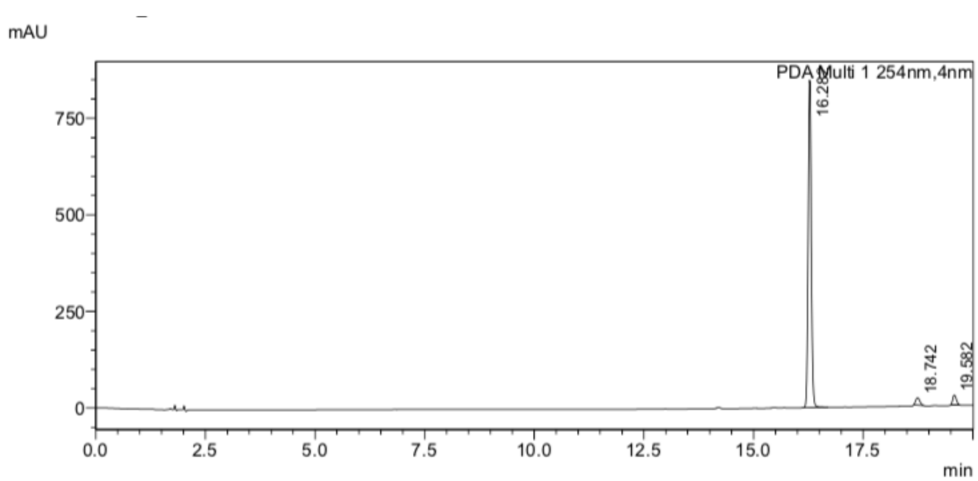


mAU
PDA Multi 1 254nm,4nm
14.431
12.479
15.188
min


Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2019-07-31T07:54:00
Modification Date 2019-07-31T07:54:42
Spectrometer Frequency 400.13
Spectral Width 8012.8
Lowest Frequency -1545.4
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
f1 (ppm)


mAU
PDA Multi 1 254nm,4nm
16.4
15.849
16.090
17.136
min


Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2019-08-10T12:40:00
Modification Date 2019-08-10T12:40:19
Spectrometer Frequency 400.13
Spectral Width 8012.8
Lowest Frequency -1545.4
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
1.06 1.04 1.01 2.05 3.00 2.01 8.05 6.15 2.01 2.11 2.15 2.25 2.33 2.10 2.21 3.00 3.02
f1 (ppm)


mAU
PDA Multi 1 254nm,4nm
16.28
18.742
19.582
min