Reversible Spatiotemporal Control of Induced Protein Degradation by Bistable PhotoPROTACs

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Content

1. Supplementary Figures

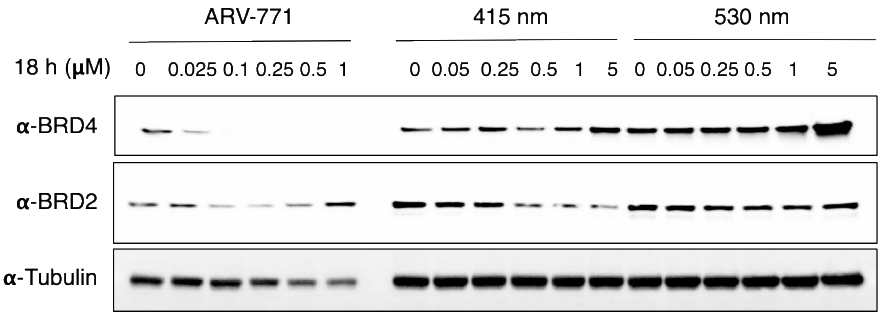


Figure S1: ARV-771 degrades both BRD2 and BRD4 whereas trans-photoPROTAC only degrades BRD2 in Ramos cells. PhotoPROTAC was irradiated using 415 nm and 530 nm LEDs for 30 minutes. Similarly, ARV-771 was also irradiated with 530 nm LED for 30 minutes before treating to cells. PhotoPROTACs were incubated with Ramos cells at the indicated concentrations. After 18h, cell lysates were analyzed for BRD protein levels by western blot analysis.

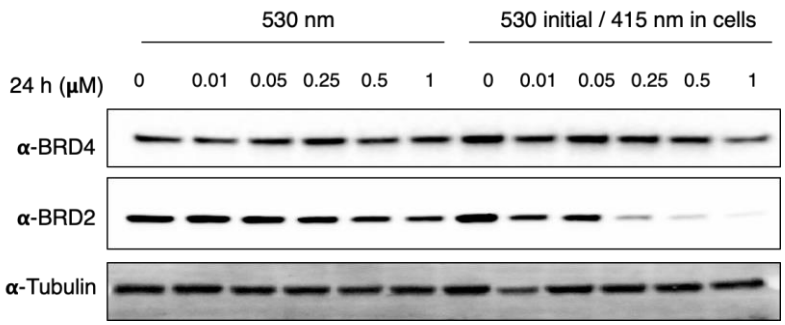


Figure S2: Temporal control of photoPROTAC activity in cells. PhotoPROTAC was initially irradiated for 30 minutes using 530 nm LED. Cis-photoPROTAC was treated to Ramos cells at different concentrations. One set of cis-photoPROTAC treated cells were incubated in dark while the other set of cells (also treated with cis-photoPROTAC) was incubated under 415 nm LED irradiation. Cells were lysed and probed for BRD protein levels.

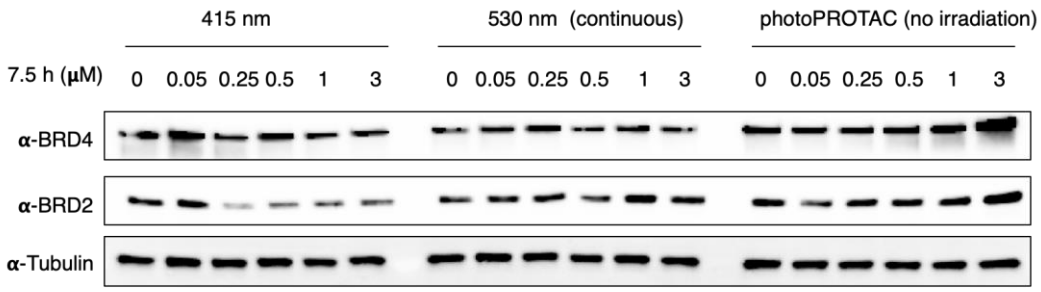


Figure S3: Continuous cellular irradiation did not yield a superior differential BRD degradation compared to BRD degradation seen in cells treated with photoPROTACs derived from an initial 30 minutes irradiation. PhotoPROTAC initially irradiated with 415 and 530 nm separately and 530 nm irradiated photoPROTAC treated cells were incubated under 530 nm LED. A separate set of cells was treated with photoPROTAC without any irradiation.

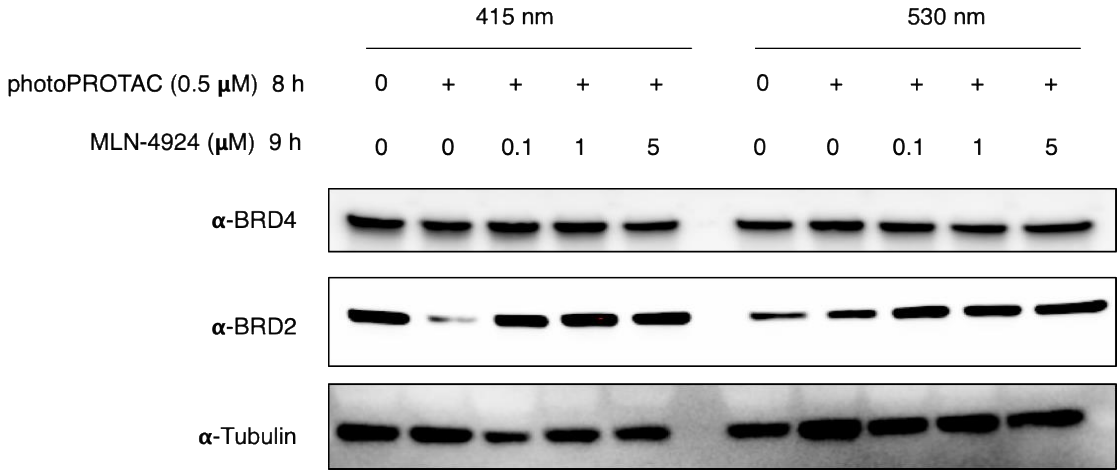
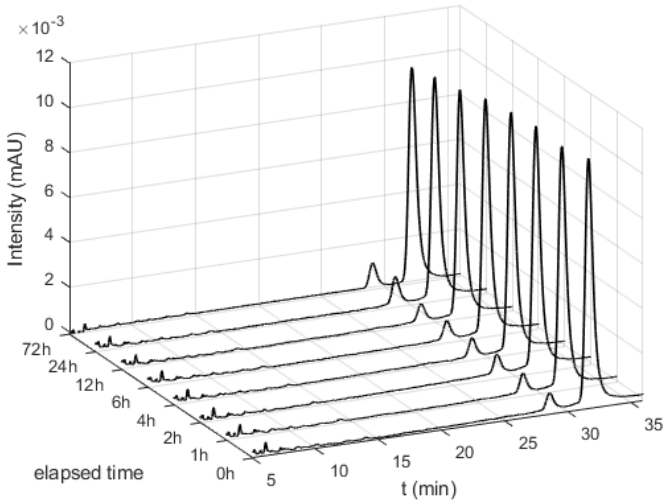


Figure S4: PhotoPROTAC mediated BRD2 degradation is proteasome-dependent. Ramos cells were pre-incubated with selective NEDD8 activating enzyme inhibitor (MLN-4924) at three different concentrations for 1h. Then cells were treated with *trans*-**photoPROTAC-1** and *cis*-**photoPROTAC-1** for another 8 h in the absence or presence of MLN-4924. Cells were lysed and western blots were probed for BRD4, BRD2 and tubulin. Active *trans*-**photoPROTAC-1** induced significant BRD2 degradation only in the absence of NEDD8 inhibitor. Inactive *cis*-**photoPROTAC-1** treatment did not induce BRD2 or BRD4 degradation.

A


×10-3
Intensity (mAU)
elapsed time
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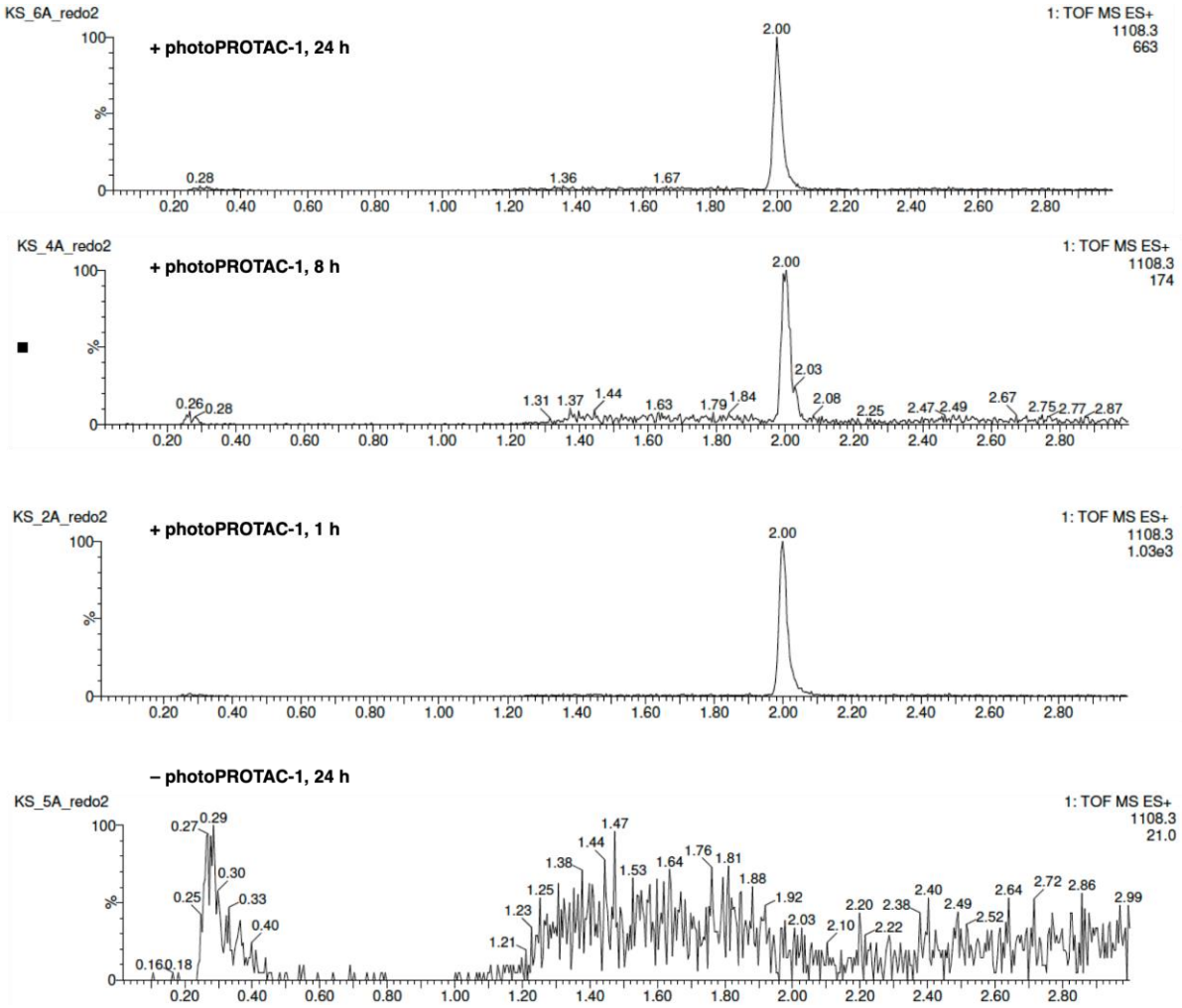
24h
12h
6h
4h
2h
1h
0h
t (min)

B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+ photoPROTAC-1, 24 h
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1108.3
663
KS_4A_redo2
+ photoPROTAC-1, 8 h
1: TOF MS ES+
1108.3
174
KS_2A_redo2
+ photoPROTAC-1, 1 h
1: TOF MS ES+
1108.3
1.03e3
– photoPROTAC-1, 24 h
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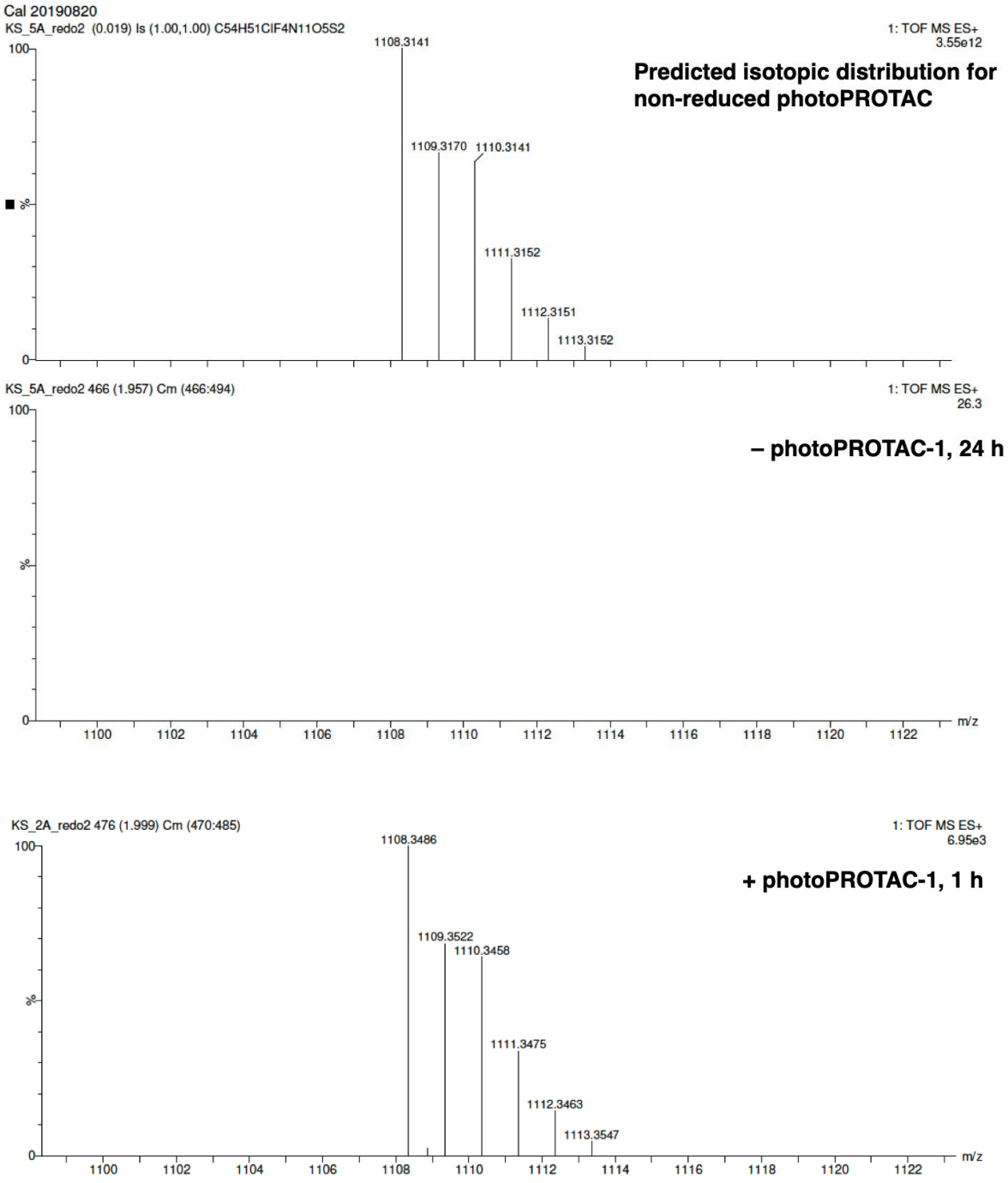
1: TOF MS ES+
1108.3
21.0

C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1: TOF MS ES+
3.55e12
100
%
0
1108.3141
1109.3170
1110.3141
1111.3152
1112.3151
1113.3152
Predicted isotopic distribution for non-reduced photoPROTAC
KS_5A_redo2 466 (1.957) Cm (466:494)
1: TOF MS ES+
26.3
100
%
0
m/z
1100
1102
1104
1106
1108
1110
1112
1114
1116
1118
1120
1122
– photoPROTAC-1, 24 h
KS_2A_redo2 476 (1.999) Cm (470:485)
1: TOF MS ES+
6.95e3
100
%
0
1108.3486
1109.3522
1110.3458
1111.3475
1112.3463
1113.3547
m/z
1100
1102
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1106
1108
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1120
1122
+ photoPROTAC-1, 1 h

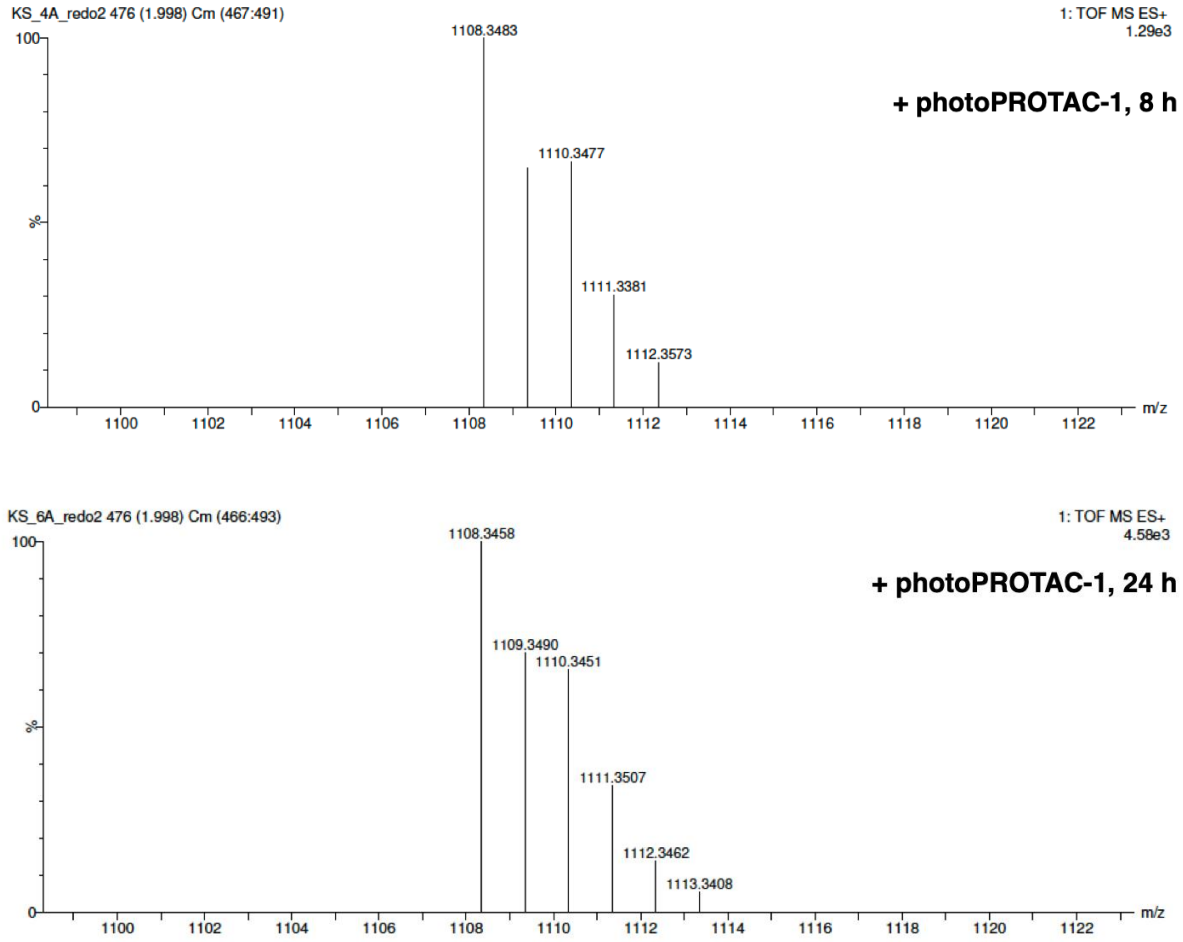


Figure S5: A: Stability test of a 50 μM stock solution of ***trans*-photoPROTAC-1** (containing up to 10% of the *cis*-isomer) in the presence of 10 mM GSH in DMSO/H_2O (1:1). The sample was analyzed vis RP-HPLC at the points of time indicated and full stability after 72 hours (3 days) was observed. **B:** Ramos cells were treated with or without **photoPROTAC-1** for different time periods and cell lysates were analyzed for reduced or non-reduced form of **photoPROTAC-1** using LC-MS. Masses corresponding to non-reduced **photoPROTAC-1** were extracted at each time point and compared to the lysates obtained from cells without **photoPROTAC-1** treatment. **C:** Isotopic distribution corresponding to the reduced form of **photoPROTAC-1** was not observed. Isotopic distribution of the non-reduced **photoPROTAC-1** indicates stability in biological system.

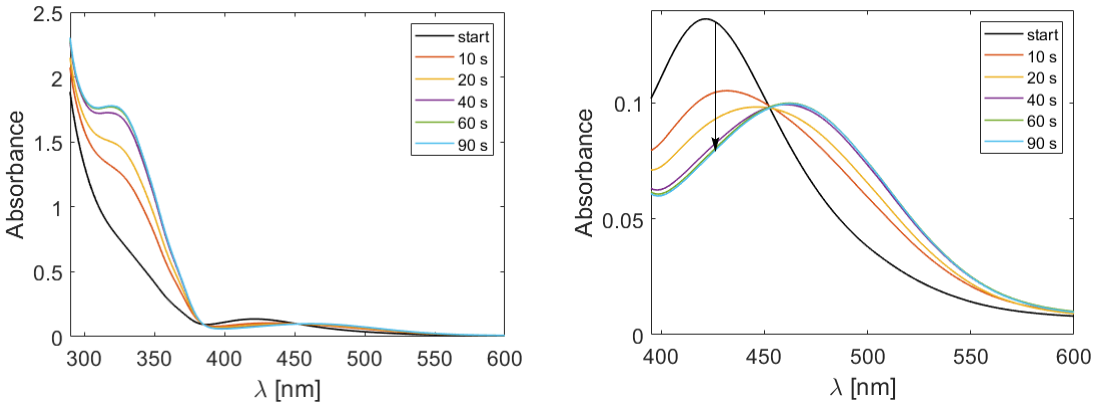


Figure S6: UV-Vis spectra of **photoPROTAC-1** at increasing time intervals of irradiation at 415 nm starting from a *cis*-enriched photostationary state (68% *cis*); left panel: UV-Vis spectra in the range of 290 to 600 nm, right panel: enlargement of the spectra in the range of 390 to 600 nm, time course indicated at 430 nm by arrow.

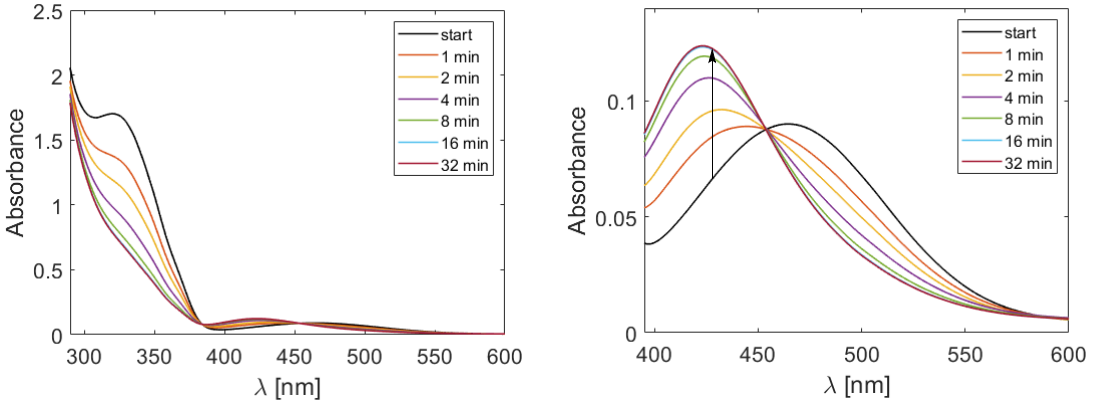


Figure S7: UV-Vis spectra of **photoPROTAC-1** at increasing time intervals of irradiation at 530 nm starting from a *trans*-enriched photostationary state (95% *trans*); left panel: UV-Vis spectra in the range of 290 to 600 nm, right panel: enlargement of the spectra in the range of 390 to 600 nm, time course indicated at 430 nm by arrow.

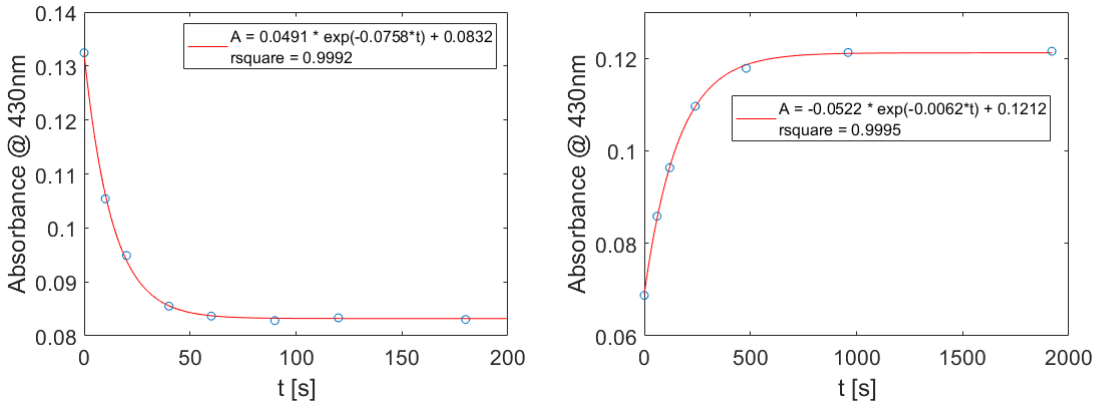


Figure S8: Time-dependence of absorbance at 430 nm for *cis*-*trans*- (left, 415 nm irradiation) and *trans*-*cis*-isomerization (right, 530 nm irradiation), respectively.

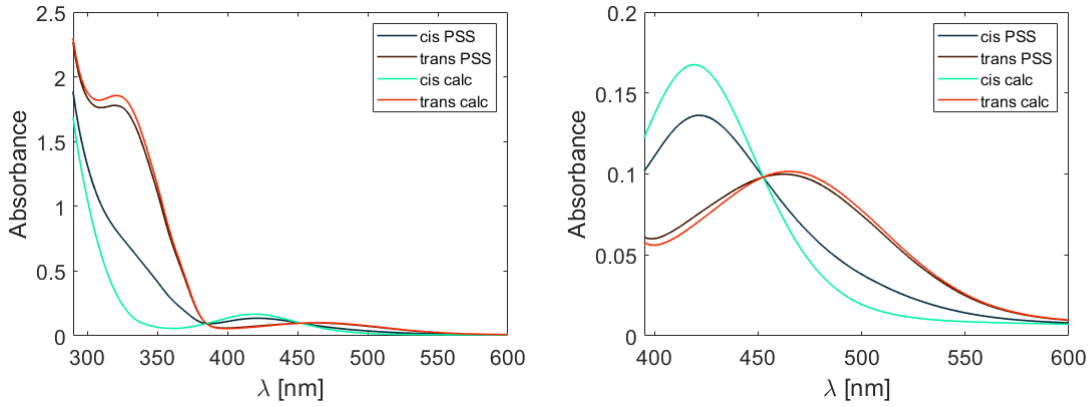


Figure S9: Calculated UV-Vis spectra of pure ***cis*-** and ***trans*-photoPROTAC-1** from PSS spectra. Left panel: UV-Vis spectra in the range of 290 to 600 nm, right panel: enlargement of the spectra in the range of 390 to 600 nm.

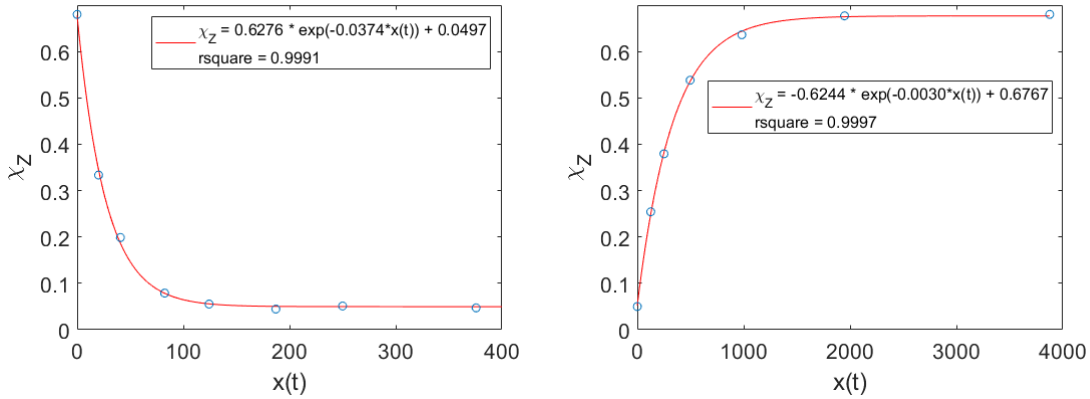


Figure S10: Plot of molar fraction of ***cis*-photoPROTAC-1** (χ_z) against the integrated photokinetic factor x(t) for *cis-trans*-(left, 415 nm irradiation) and *trans-cis*-isomerization (right, 530 nm irradiation), respectively. From the respective fit functions, quantum yields are determined – as described in S2.9.

2. Materials and Methods

2.1 General Synthetic Methods. Unless otherwise noted, all reactions were carried out under N_2 atmosphere, and all reagents were purchased from commercial suppliers (ABCR, ACROS, Sigma Aldrich, Fluka, TCI, Strem, Alfa, Combi-Blocks or Fluorochem) and used without further purification. Anhydrous solvents over molecular sieves were purchased from Acros and used as received. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 TLC glass plates and visualized with 254 nm light and potassium permanganate or ceric ammonium molybdate staining solutions followed by heating. Organic solutions were concentrated by rotary evaporation at 40 °C. Purification of reaction products was carried out by flash chromatography using Sigma Aldrich silica 230-400, 60Å under 0.3–0.5 bar overpressure.

2.2 Safety Statement. No unexpected or unusually high safety hazards were encountered. When working with LEDs, direct eye contact with the light source should be avoided to prevent eye damage.

2.3 Analysis of Synthetic Compounds. ^{1}H NMR spectra were recorded on a Bruker AVIII HD 500 MHz and 400 spectrometers as well as Bruker Neo 500 MHz and 400 MHz spectrometers, and are reported in ppm with the solvent resonance as the reference ($CDCl_3$ at 7.26 ppm, CD_2Cl_2 at 5.32 ppm, CD_3OD at 3.31 ppm, DMSO-d_6 at 2.50 ppm, $(CD_3)_2CO$ at 2.05 ppm, CD_3CN at 1.94 ppm). Peaks are reported as "(s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or unresolved, br = broad signal, coupling constant(s) in Hz, integration)". ^{13}C NMR spectra were recorded with 1H-decoupling on Bruker AVIII HD 125 MHz and 100 MHz spectrometers as well as Bruker Neo 125 MHz and 100 MHz spectrometers, and are reported in ppm with the solvent resonance as the reference unless noted otherwise ($CDCl_3$ at 77.16 ppm, CD_2Cl_2 at 53.84 ppm, CD_3OD at 49.00 ppm, DMSO-d_6 at 39.52 ppm, $(CD_3)_2CO$ at 29.84 ppm, CD_3CN at 1.32 ppm). Infrared spectra were recorded neat on a Perkin-Elmer Spectrum Two FT-IR spectrometer. The peaks are reported as absorption maxima (cm^{-1}). High resolution mass spectrometric data were obtained at the mass spectrometry service operated by the Laboratory of Organic Chemistry at ETHZ on VG-TRIBRID for electron impact ionization (EI), Varian IonSpec Spectrometer for electrospray ionization (ESI), or IonSpec Ultima Fourier Transform Mass Spectrometer for matrix-assisted laser desorption/ionization (MALDI) and are reported as (m/z).

2.4 Photochemical Characterization. UV-Vis spectra were obtained in the specified solvent at the respective concentration on a Jasco V-630 spectrometer. Photoswitch samples were irradiated for the indicated time period at either 415 nm (M415F3 LED) or 530 nm (M530F2 LED) with fiber-coupled LED equipment purchased from ThorLabs. The LEDs were powered by a LEDD1B-Driver and were used at full power while directing the beam of light with an optical patch cable (400 UMT custom MUC). The respective cis/trans isomers were separated using a gradient of 60% to 40% H_2O in CH_3CN

(containing 0.1% formic acid) on a Reprosil Chiral-TG 5 μm column (250 x 4.6 mm) in an analytical RP-HPLC setup (Waters e2965 separations module equipped with a 2998 PDA detector). The cis/trans ratios in the respective photostationary states were quantified by integration of the HPLC-chromatogram at the isosbestic wavelength of 275 nm in CH_3CN/H_2O (1:1).

2.5 Cell Culture. Ramos cells were grown in complete RPMI1640 cell culture medium supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% Penicillin and Streptomycin. Ramos suspension cells were grown in cell culture flasks under 5% CO_2 at 37°C in a humidified incubator. Prior to the photoPROTAC treatment, cells (3 X 10^6) were added to each well in 6-well plates with 2 mL of cell culture medium. To assess the BRD protein degradation by photoPROTACs, pre-irradiated photoPROTACs were treated with varying concentrations for indicated time points. For proteasomal pathway-dependent experiments, cells were pre-treated with MLN-4924 for 1 hour prior to photoPROTAC treatment. After initial irradiation, all the steps were performed under dark conditions until preparing the cell lysates for boiling. After desired time points, cells were harvested by centrifugation of Ramos cells at 4000 rpm for 4 minutes. Cells were washed once with 1 mL of prechilled 1X phosphate buffer saline (PBS) and cell pellets either store at -80°C or continued for the cell lysis. Cell lysates were prepared by incubating cell pellet with 80 μL of ThermoFisher RIPA lysis buffer containing 1X protease inhibitor cocktail for 30 minutes on ice. Then cell debris were pelleted down by high speed centrifugation at 14000 rpm for 12 minutes at 4°C. Clear supernatant was transferred to fresh Eppendorf tubes and protein concentrations were analyzed by BCA protein assay kit.

2.6 Irradiation of photoPROTAC. PhotoPROTAC stock solution was diluted in DMSO to get 100 μM working solution. Then, working solution was divided into two separate tubes for individual irradiation by 415 nm and 530 nm LEDs to obtain *trans* and *cis* isomers of photoPROTAC respectively. During irradiation process all the steps were carried out in a dark room. Two tubes were exposed to 415 nm and 530 nm light for 30 minutes including several vortexing steps during the irradiation process. During irradiation process all the steps were carried out in a dark room. To test the reversibility of the photoPROTAC, the second irradiation step was performed after the initial irradiation. Following the initial irradiation by 4125 nm or 530 nm LEDs, irradiated photoPROTACs were divided into two separate tubes. One tube was saved for the cellular treatment without second irradiation step and the other tube containing 415 nm or 530 nm irradiated photoPROTAC was subjected to the second irradiation (for another 30 minutes) by 530 nm or 415 nm to reverse the initial photostationary state (415/530 nm and 530/415 nm). Prior to the cell culture treatment, 100 μM working solutions were further a serially diluted in DMSO to obtain 1, 5, 25, 50 μM stocks and equal volumes from each isomer

was added to 2 mL of cell culture medium and incubated in dark for indicated time points. To test the temporal controllability of photoPROTACs, two sets of Ramos cells were treated with the same PROTAC isomer (trans/415 nm) and one set of cells was incubated in dark while the other set of cells was incubated under the other 530 nm LED or vice versa.

2.7 Western Blot Analysis. After performing the BCA protein assay (ThermoFisher), all the cell lysates were combined with gel loading SDS buffer and boiled for 5 minutes. Cell lysates were then briefly centrifuged before loading and protein separation by SDS PAGE. After running the SDS gel, proteins were transferred to a PVDF membrane and incubated with primary antibodies against BRD4 (Bethyl Laboratories-A700-004,1:1000 dilution), BRD2 (Bethyl Laboratories-A700-008, 1:1000 dilution) and Tubulin (Sigma-16-232) overnight at 4°C with gentle shaking. All primary and secondary antibodies were prepared in 1X TBST with 5% skim milk (0.01% Tween-20, 25 mM Tris buffer pH-7.4, 150 mM NaCl). PVDF membranes were washed three times with 1X TBST with 5 minutes incubation at room temperature. Secondary antibodies conjugated to horse reddish peroxidase enzyme (HRP) were diluted at 1:10000 in 1X TBST with 5% skim milk and membranes were incubated for 1 h at room temperature with gentle shaking. After three time washing with 1X TBST, protein bands were visualized using Bio-Rad ChemiDoc system by adding freshly prepared 1:1 HRP substrate mixture.

2.8 GSH Stability Test. In a 2 ml amberized vial, 750 μl of a 100 μM ***trans*-photoPROTAC-1** stock solution in DMSO were combined with 750 μl of a 20 mM reduced glutathione stock solution in H_2O to give a 1.5 ml solution in DMSO/H_2O (1:1) of 50 μM ***trans*-photoPROTAC-1** and 10 mM GSH. The mixture was purged with nitrogen for 5 minutes and, subsequently, the sample was analyzed by HPLC under the conditions specified in **2.3** at the points of time indicated in **Figure S5A**. The observed fully retained stability of the *ortho*-F_4-azobenzene derivative after an incubation with GSH for 3 days is in line with the observation in a previous report.[1]

2.9 Quantum Yield Measurement. A 100 μM stock solution of ***cis*-** or ***trans*-photoPROTAC-1** (DMSO) in their respective PSS obtained after irradiation with 530 nm (68% *cis*) and 415 nm (95% *trans*, see **Fig. 3C**) was irradiated for increasing time periods and after each interval, UV-Vis spectra were recorded (**Figure S6, S7**). Time-dependent absorbance was subsequently analyzed at 430 nm (**Figure S8**). The respective absorbances $A_\lambda^{E/Z}$ of both isomers at any wavelength were calculated from isomer composition and UV-Vis spectra in the respective PSS, allowing to calculate individual spectra of ***cis*-** and ***trans*-photoPROTAC-1** (**Figure S9**) by solving following linear equation system (with A_λ = total absorbance at wavelength λ; χ = molar fraction; $A_\lambda^{E/Z} = \epsilon_\lambda^{E/Z} c_0 l$ = absorbance of either isomer in concentration c_0 at wavelength λ):

$$A_\lambda = \chi_\mathrm{E} * A_\lambda^\mathrm{E} + \chi_\mathrm{Z} * A_\lambda^\mathrm{Z} \quad (1)$$

$$A_\lambda = (1 - \chi_\mathrm{Z}) * A_\lambda^\mathrm{E} + \chi_\mathrm{Z} * A_\lambda^\mathrm{Z} \quad (2)$$

$$A_\lambda^{\text{PSS @ 530 nm}} = 0.32 * A_\lambda^\mathrm{E} + 0.68 * A_\lambda^\mathrm{Z} \quad (3)$$

$$A_\lambda^{\text{PSS @ 415 nm}} = 0.95 * A_\lambda^\mathrm{E} + 0.05 * A_\lambda^\mathrm{Z} \quad (4)$$

From (2) follows equation (5) which allows to calculate χ_Z at any given time t and wavelength λ.

$$\chi_\mathrm{Z}(t) = \frac{A_\lambda(t) - A_\lambda^\mathrm{E}}{A_\lambda^\mathrm{Z} - A_\lambda^\mathrm{E}} \quad (5)$$

The quantum yields were then calculated based on a kinetic model (6) of an equilibrium between *cis*- and *trans*-isomer.[2] The thermal back reaction was neglected since for *o*-F_4-azobenzene derivatives it is especially slow. The respective rate constants $k_{\mathrm{EZ/ZE}}$ are given by (7)[3,4] (with I_0 = intensity of excitation light source; $A_\lambda(t)$ = absorbance at (arbitrary) wavelength λ as function of time t; ϕ = quantum yield; V = volume of irradiated solution; c_0 = initial concentration of isomer mixture; since $A_{\lambda_\mathrm{irr}}^{\mathrm{E/Z}}$ is determined from the same set of data, the optical pathway length l is not included in (7) as it is already contained in $A_\lambda^{\mathrm{E/Z}}$). In the photostationary state $\frac{d\chi_\mathrm{Z}}{dt} = 0$, resulting in (8).

$$\frac{d\chi_\mathrm{Z}}{dt} = k_\mathrm{EZ} * \chi_\mathrm{E} - k_\mathrm{ZE} * \chi_\mathrm{Z} \quad (6)$$

$$k_\mathrm{EZ/ZE} = \frac{I_0 * \phi_\mathrm{EZ/ZE} * A_{\lambda_\mathrm{irr}}^\mathrm{E/Z}}{V * c_0} * \frac{1 - 10^{-A_\lambda(t)}}{A_\lambda(t)} \quad (7)$$

$$\frac{\phi_\mathrm{EZ}}{\phi_\mathrm{ZE}} = \frac{\chi_{\mathrm{Z},\infty} * A_{\lambda_\mathrm{irr}}^\mathrm{Z}}{(1 - \chi_{\mathrm{Z},\infty}) * A_{\lambda_\mathrm{irr}}^\mathrm{E}} \quad (8)$$

By inserting (7) and (8) in (6), (9a) and (9b) are obtained. Within (9a) and (9b) $B_\mathrm{E/Z}$ can be defined as constant factors, giving (10a) and (10b).

$$\frac{d\chi_\mathrm{Z}}{dt} = \frac{I_0 * \phi_\mathrm{EZ} * A_{\lambda_\mathrm{irr}}^\mathrm{E}}{V * \chi_{\mathrm{Z},\infty} * c_0} * \frac{1 - 10^{-A_\lambda(t)}}{A_\lambda(t)} * (\chi_{\mathrm{Z},\infty} - \chi_\mathrm{Z}) \quad (9a)$$

$$\frac{d\chi_\mathrm{Z}}{dt} = \frac{I_0 * \phi_\mathrm{ZE} * A_{\lambda_\mathrm{irr}}^\mathrm{Z}}{V * (1 - \chi_{\mathrm{Z},\infty}) * c_0} * \frac{1 - 10^{-A_\lambda(t)}}{A_\lambda(t)} * (\chi_{\mathrm{Z},\infty} - \chi_\mathrm{Z}) \quad (9b)$$

$$B_\mathrm{E} = \frac{I_0 * \phi_\mathrm{EZ} * A_{\lambda_\mathrm{irr}}^E}{V * \chi_{\mathrm{Z},\infty} * c_0} \quad (10a)$$

$$B_\mathrm{Z} = \frac{I_0 * \phi_\mathrm{ZE} * A_{\lambda_\mathrm{irr}}^\mathrm{Z}}{V * \left(1 - \chi_{\mathrm{Z},\infty}\right) * c_0} \quad (10b)$$

Solving differential equation (9) results in general solution (11). Under the boundary condition of $\chi_\mathrm{Z}(t_0) = \chi_{\mathrm{Z},0}$, c_1 is identified as $c_1 = \chi_{\mathrm{Z},0} - \chi_{\mathrm{Z},\infty}$. Lastly, definition of integrated photokinetic factor (12) yields (13). By plotting $\chi_\mathrm{Z}(x(t))$ vs. $x(t)$ and finding an appropriate fit (**Figure S10**), factors $B_\mathrm{E/Z}$ are extracted from the fitting parameters and quantum yields $\phi_\mathrm{EZ/ZE}$ can be calculated according to

(10a) and (10b). The intensity I_0 of the LED light sources is obtained as photon flux in mol/s via (14)[5] from technical details provided by the LED-supplier (where λ_{irr} = irradiation peak wavelength of LED; P_{LED} = radiant power of LED (21.3 mW for 415 nm LED, 9.9 mW for 530 nm LED); h = Planck constant; c = speed of light; N_A = Avogadro constant).

$$\chi_Z(t) = c_1 * \exp\left(-B \int_{t_0}^{t} \frac{1 - 10^{-A_\lambda(t)}}{A_\lambda(t)} dt\right) + \chi_{Z,\infty} \quad (11)$$

$$x(t) = \int_{t_0}^{t} \frac{1 - 10^{-A_\lambda(t)}}{A_\lambda(t)} dt \quad (12)$$

$$\chi_Z(x(t)) = (\chi_{Z,0} - \chi_{Z,\infty}) * \exp(-B * x(t)) + \chi_{Z,\infty} \quad (13)$$

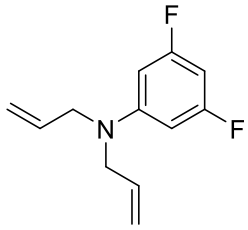
$$I_0 = \frac{\lambda_{irr} P_{LED}}{hcN_A} \quad (14)$$

Following this procedure, the quantum yields of the respective isomerization reactions were determined at both irradiation wavelengths as mean of three measurements (standard deviation < 0.03):

	415 nm	**530 nm**
$\boldsymbol{\phi_{EZ}}$	0.09	0.29
$\boldsymbol{\phi_{ZE}}$	0.66	0.62

3. Synthetic Procedures

N,N-diallyl-3,5-difluoroaniline (5)



3,5-Difluoroaniline (1.05 g, 8.13 mmol, 1.00 equiv) was dissolved in EtOH (16.3 ml) and H_2O (4.1 ml, 0.4 M in total). Na_2CO_3 (1.03 g, 9.76 mmol, 1.20 equiv) and allyl bromide (1.69 ml, 19.5 mmol, 2.40 equiv) were added to the reaction mixture which was stirred at reflux for 3 hours. Then, EtOH was removed in vacuo and the residual aq. phase was extracted with CH_2Cl_2 (4x). The combined org. layers were washed with brine and dried over Na_2SO_4. The crude product was further purified by column chromatography (4% CH_2Cl_2 in hexanes) to afford **5** as a clear oil (860 mg, 4.11 mmol, 51%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.19 – 6.07 (m, 3H), 5.86 – 5.76 (m, 2H), 5.22 – 5.12 (m, 4H), 3.90 – 3.87 (m, 4H).

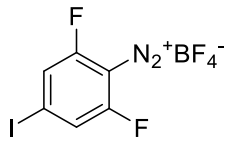
^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.3 (dd, J = 242, 16 Hz), 150.9 (t, J = 13 Hz), 132.9, 116.6, 95.1 (dd, J = 21, 9 Hz), 91.5 (t, J = 26 Hz), 53.0.

^{19}F NMR (377 MHz, $CDCl_3$) δ = -111.0.

IR: 3088, 2912, 2874, 1632, 1580, 1696, 1467, 1179, 1171, 1111, 990, 969, 922, 815, 670.

ESI-HRMS: calcd. for $C_{12}H_{14}F_2N_1$ $[M+H]^+$ 210.1089, found 210.1091.

2,6-difluoro-4-iodobenzenediazonium tetrafluoroborate (4)



2,6-Difluoro-4-iodoaniline **3** (1.00 g, 3.92 mmol, 1.00 equiv) was dissolved in EtOH (0.98 ml, 4 M). While cooling to -5 °C, HBF_4 was added to the reaction mixture. Still at -5 °C, isoamyl nitrite (0.591 ml, 4.39 mmol, 1.12 equiv) was added dropwise over the course of 5 minutes to the reaction mixture. After another 5 minutes, the mixture was allowed to warm to room temperature and stirred at this temperature for another 30 minutes. The mixture was filtered and washed with EtOH and ice-cold Et_2O to afford **4** as a grey solid powder (540 mg, 1.33 mmol, 34%).

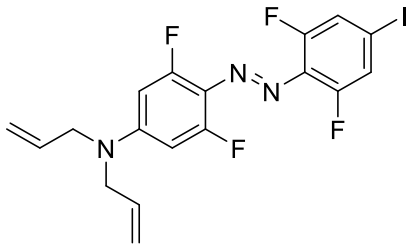
^{1}H NMR (400 MHz, CD_3CN) δ = 8.19 – 8.11 (m, 2H).

^{13}C NMR (400 MHz, CD_3CN) δ = 159.8 (d, J = 284 Hz), 126.7 – 126.4 (m), 119.2, [C-N_2^+ not visible due to broad signal].

^{19}F NMR (377 MHz, CD_3CN) δ = -99.2, -150.5, -150.6.

IR: 3084, 2265, 1590, 1438, 1290, 1179, 1220, 1067, 1052, 849, 579.

(E)-N,N-diallyl-4-((2,6-difluoro-4-iodophenyl)diazenyl)-3,5-difluoroaniline (6)



In a flame-dried flask, *n*-BuLi (1.5 M in hexanes, 1.07 ml, 1.00 equiv) was added to a solution of TMEDA (245 μl, 1.63 mmol, 1.00 equiv) in anhydrous THF (1.6 ml, 1.0 M) at – 78 °C. To this mixture was added N,N-diallyl-3,5-difluoroaniline **5** (340 mg, 1.63 mmol, 1.00 equiv) via syringe and the mixture was left to stir for 1 hour during which the mixture was allowed to warm up to -50 °C. Meanwhile, in another flame-dried flask diazonium salt **4** (575 mg, 1.63 mmol, 1.00 equiv) was suspended in anhydrous THF (1.6 ml, 1.0 M). Then, the lithiated mixture was cooled back to -78 °C and was quickly added to the diazonium salt suspension at -78 °C by cannulation. Residual lithiated material was rinsed by anhydrous THF (2x 1.5 ml) followed by cannulation to the reaction mixture. The obtained dark red mixture was stirred for another hour during which it warmed to room temperature. The mixture was then quenched by addition of sat. aq. $NaHCO_3$ and diluted with EtOAc and H_2O. The phases were separated, and the aq. phase was extracted with EtOAc (3x). The combined org. layers were washed with brine and dried over Na_2SO_4. After concentrating under reduced pressure, the crude product was further purified by flash column chromatography (10% EtOAc in hexanes) to afford **6** (260 mg, 0.547 mmol, 34%) as a red oil.

Rf = 0.67 (30% EtOAc in hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39 – 7.35 (m, 2H), 6.29 – 6.25 (m, 2H), 5.87 – 5.78 (m, 2H), 5.27 – 5.15 (m, 4H), 4.00 – 3.96 (m, 4H).

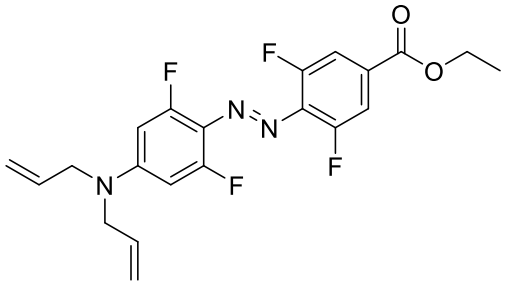
^{13}C NMR (126 MHz, $CDCl_3$) δ = 158.7 (dd, J = 260, 8 Hz), 155.1 (dd, J = 263, 5 Hz), 152.3 (d, J = 29 Hz), 132.6, 131.61, 122.8, 122.41 – 121.67 (m), 117.4, 95.7 (dd, J = 26, 2 Hz), 90.7 (t, J = 10 Hz), 53.1.

^{19}F NMR (471 MHz, $CDCl_3$) δ = -116.3, -121.5.

IR: 2926, 2853, 1626, 1552, 1513, 1389, 1179, 1043, 927, 840, 818.

ESI-HRMS: calcd. for $C_{18}H_{15}F_4I_1N_3$ $[M+H]^+$ 476.0241, found 476.0241.

ethyl (E)-4-((4-(diallylamino)-2,6-difluorophenyl)diazenyl)-3,5-difluorobenzoate (7)



Iodoaniline **6** (93.0 mg, 196 μmol, 1.00 equiv) and $Pd(PPh_3)_2Cl_2$ (2.75 mg, 3.91 μmol, 2.00 mol%) were transferred into a flame-dried two-necked flask. The mixture was flushed with 3 balloons of CO and kept under an atmosphere of CO. Then, a solution of NaOEt (16.8 mg, 235 μmol, 1.2 equiv) in EtOH (1.89 ml, 0.1 M) was added to the reaction mixture and, to enhance solubility, DMF (0.285 ml) was added as a cosolvent. The reaction mixture was stirred for 4 hours at room temperature. CO was blown off under a stream of N_2 and the reaction mixture was quenched by addition of sat. aq. NH_4Cl and diluted with EtOAc. Phases were separated, and the aq. phase was extracted with EtOAc (3x) and the combined organic layers were washed with brine and dried over Na_2SO_4. The crude product was further purified by flash column chromatography (10% EtOAc in hexanes) to afford **7** as a dark red oil (75.0 mg, 180 μmol, 91%).

Rf = 0.60 (30% EtOAc in hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.69 – 7.64 (m, 2H), 6.31 – 6.26 (m, 2H), 5.87 – 5.79 (m, 2H), 5.28 – 5.15 (m, 4H), 4.40 (q, J = 7.1 Hz, 2H), 4.01 – 3.97 (m, 4H), 1.41 (t, J = 7.1 Hz, 3H).

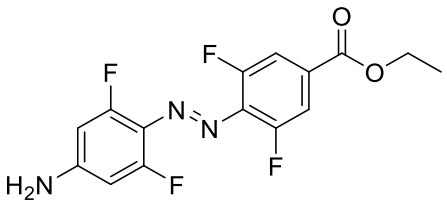
^{13}C NMR (126 MHz, $CDCl_3$) δ = 164.4 (t, J = 3.0 Hz), 158.8 (dd, J = 261, 8 Hz), 154.9 (dd, J = 258, 5 Hz), 152.7 (t, J = 14 Hz), 135.9 (t, J = 11 Hz), 131.5, 130.7 (t, J = 9 Hz), 122.9 (t, J = 9 Hz), 117.5, 113.8 (dd, J = 20, 6 Hz), 95.7 (dd, J = 26, 2 Hz), 61.9, 53.2, 14.4.

^{19}F NMR (471 MHz, $CDCl_3$) δ = -115.7, -121.9.

IR: 3088, 2985, 2935, 1722, 626, 1552, 1428, 1386, 1331, 1230, 1178, 1044, 929, 818, 769.

ESI-HRMS: calcd. for $C_{21}H_{20}F_4N_3O_2$ $[M+H]^+$ 422.1486, found 422.1489.

ethyl (E)-4-((4-amino-2,6-difluorophenyl)diazenyl)-3,5-difluorobenzoate (8)



In a sealed tube under N_2, N,N-diallylamine **7** (18.0 mg, 43.0 μmol, 1.00 equiv) and $RuClH(CO)(PPh_3)_3$ (0.410 mg, 0.430 μmol, 1.00 mol%) were dissolved in anhydrous CH_2Cl_2 (0.21 ml, 0.2 M). The reaction mixture was heated to 60 °C for 4 hours. At room temperature, $NH_2OH{\cdot}HCl$ (44.5 mg, 0.641 μmol 15.0 equiv) and NEt_3 (30.0 μl, 214 μmol, 5.00 equiv) were added to the reaction mixture which was heated to 80 °C for another 4 hours. Then, after cooling to room temperature, the reaction mixture was quenched by addition of sat. aq. $NaHCO_3$ and the mixture was diluted with EtOAc and H_2O. Phases were separated, and the aq. phase was extracted with EtOAc (3x) and the combined org. layers were washed with brine and dried over Na_2SO_4. The crude product was further purified by flash column chromatography (30% EtOAc in hexanes) to afford **8** as an orange oil (8.0 mg, 23 μmol, 55%).

Rf = 0.35 (40% EtOAc in hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.70 – 7.66 (m, 2H), 6.31 – 6.26 (m, 2H), 4.43 (s, 2H), 4.40 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H).

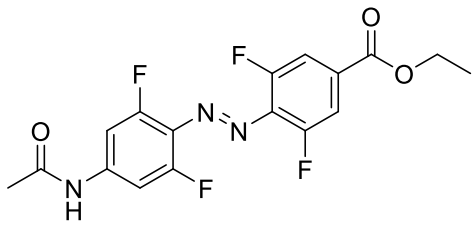
^{13}C NMR (126 MHz, $CDCl_3$) δ = 164.3, 158.7 (dd, J = 263, 7 Hz), 155.0 (dd, J = 258, 5 Hz), 151.7 (t, J = 15 Hz), 135.5 (t, J = 11 Hz), 131.3 (t, J = 9 Hz), 124.2, 113.8 (dd, J = 20, 6 Hz), 98.1 (dd, J = 24, 2 Hz), 77.4, 14.4.

^{19}F NMR (471 MHz, $CDCl_3$) δ = -116.1, -121.6.

IR: 3390, 1712, 1637, 1567, 1437, 1363, 1332, 1241, 1175, 1041, 768.

ESI-HRMS: calcd. for $C_{15}H_{12}F_4N_3O_2$ $[M+H]^+$ 342.0860, found 342.0862.

ethyl (E)-4-((4-acetamido-2,6-difluorophenyl)diazenyl)-3,5-difluorobenzoate (9)



Aniline **9** (6.00 mg, 18.0 μmol, 1.00 equiv) was dissolved in anhydrous CH_2Cl_2 (90 μl, 0.2 M) and while stirring at room temperature, acetic anhydride (1.80 μl, 19.0 μmol, 1.10 equiv) was added to the reaction mixture. After 24 h, more acetic anhydride (1.00 μl, 10.6 μmol, 0.60 equiv) was added to the reaction mixture and after a total of 48 hours the reaction mixture was quenched by addition of sat. aq. $NaHCO_3$. The mixture was diluted with EtOAc and H_2O and the phases were separated. The aq. phase was extracted with EtOAc and the combined org. layers were washed with brine and dried over Na_2SO_4. The crude product was further purified by flash column chromatography (30% to 60% EtOAc in hexanes) to afford the product as an orange solid (5.0 mg, 13.0 mmol, 74%).

Rf = 0.46 (60% EtOAc in hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.74 – 7.69 (m, 2H), 7.38 (s, 1H), 7.38 – 7.34 (m, 2H), 4.42 (q, J = 7.1 Hz, 2H), 2.24 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H).

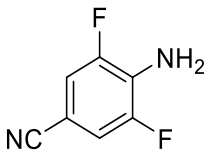
^{13}C NMR (126 MHz, $CDCl_3$) δ = 168.6, 164.0, 157.0 (dd, J = 262, 6 Hz), 155.0 (dd, J = 261, 4 Hz), 142.0, 134.9, 132.6, 127.8, 114.1 – 113.8 (m), 103.4 – 103.1 (m), 62.2, 25.0, 14.4.

^{19}F NMR (471 MHz, $CDCl_3$) δ = -116.7, -120.7.

IR: 3348, 3113, 2925, 2854, 1718, 1690, 1628, 1598, 1540, 1482, 1425, 1372, 1333, 1238, 1154, 1052, 850, 770.

ESI-HRMS: calcd. for $C_{17}H_{14}F_4N_3O_3$ $[M+H]^+$ 384.0966, found 384.0971.

4-amino-3,5-difluorobenzonitrile (S1)[6]



2,6-Difluoro-4-iodoaniline (4.00 g, 15.7 mmol, 1.00 equiv) and CuCN (2.25 g, 25.1 mmol, 1.60 equiv) were suspended in NMP (31.4 ml, 0.5 M) and the mixture was heated to 180 °C for 7 hours. After cooling to room temperature, ethylenediamine (3.40 ml, 50.2 mmol, 3.20 equiv) was added and the mixture was poured onto water and was further diluted with EtOAc. The phases were separated, and the organic layers were washed with 10% ethylenediamine in H_2O and H_2O. The organic phase was dried over Na_2SO_4 and filtered through a pad of Celite. The crude product was further purified by flash column chromatography (dry loading on Celite, 20 to 40% EtOAc in hexanes) to afford **S1** as a colorless solid (2.2 g, 14.3 mmol, 91%).

Rf = 0.34 (20% EtOAc in hexanes).

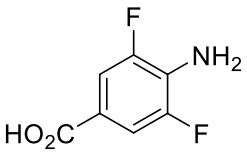
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 – 7.09 (m, 2H), 4.27 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 150.53 (dd, J = 243.5, 8.9 Hz), 129.62 (t, J = 15.8 Hz), 117.95 (t, J = 3.3 Hz), 115.72 – 115.25 (m), 98.34 (t, J = 11.1 Hz).

^{19}F NMR (377 MHz, $CDCl_3$) δ = -131.3.

IR: 3485, 3367, 3232, 2248, 2230, 1642, 1586, 1532, 1448, 1353, 1278, 1149, 1122, 1149, 972, 958, 869, 614.

4-amino-3,5-difluorobenzoic acid (S2)[7]



Nitrile **S1** (1.04 g, 6.75 mmol, 1.00 equiv) was suspended in 1 M aq. NaOH (35.1 ml, 5.20 equiv) and the mixture was stirred under reflux for 1 hour. After cooling to room temperature, the reaction mixture was acidified to pH 2 by addition of 5 M HCl. The resulting suspension was diluted with EtOAc and the phases were separated. The aq. phase was extracted with EtOAc (3x) and the combined org layers were washed with 1 M HCl and brine and dried over Na_2SO_4. After concentrating under reduced pressure, **S2** was obtained as an off-white solid (1.26 g, 6.01 mmol, 89%) which was used without further purification in the subsequent step.

Rf = 0.26 (80% EtOAc in hexanes).

^{1}H NMR (400 MHz, CD_3OD) δ = 7.50 – 7.40 (m, 2H), 4.93 (s, 3H).

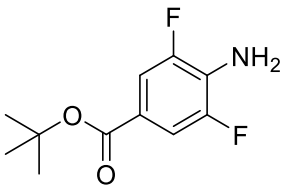
^{13}C NMR (101 MHz, CD_3OD) δ = 168.7 (t, J = 3.3 Hz), 151.8 (dd, J = 239.5, 8.9 Hz), 131.9 (t, J = 16.5 Hz), 118.0 (t, J = 8.0 Hz), 113.74 – 113.32 (m).

^{19}F NMR (377 MHz, CD_3OD) δ = -133.4.

IR: 3490, 3386, 2980, 1692, 1636, 1590, 1455, 1421, 1344, 1281, 1252, 1150, 966, 955, 887, 765, 720, 560, 458.

ESI-HRMS: calcd. for $C_7H_4F_2NO_2$ $[M-H]^-$ 172.0216, found 172.0217.

***tert*-butyl 4-amino-3,5-difluorobenzoate (10)**



Acid **S2** (1.26 g, 6.01 mmol, 1.00 equiv) was dissolved in anhydrous THF (20.0 ml, 0.3 M) and DMF (0.931 ml, 12.0 mmol, 2.00 equiv) was added to the reaction mixture. Then, $(COCl)_2$ (1.05 ml, 12.0 mmol, 2.00 equiv) was added dropwise to the reaction mixture. The reaction mixture was left to stir for 30 minutes. Then, KO*t*-Bu (1 M in THF, 36.1 ml, 6.00 equiv) was added slowly while cooling to 0 °C. The resulting brown suspension was quenched by addition of H_2O and diluted with EtOAc. The phases were separated, and the aq. phase was extracted with EtOAc (4x). The combined org layers were washed with brine and dried over Na_2SO_4. Analysis of the crude NMR indicated a mixture of amidine and formamido species.

In a pressure tube, this mixture was suspended in EtOH (3.0 ml, 0.2 M) and N,N'-dimethylethane-1,2-diamine (2.91 ml, 27.0 mmol, 4.5 equiv) was added. The pressure tube was sealed, and the reaction mixture was heated to 110 °C for 12 hours. Then, EtOH was removed under reduced pressure and the residue was dissolved in H_2O, brine and EtOAc. Phases were separated, and the aq. phase was extracted with EtOAc. The combined org. layers were washed with brine, dried over Na_2SO_4 and concentrated. The crude product was further purified by column chromatography (dry loading on Celite, 10% EtOAc in hexanes) to afford **10** as a yellow solid (675 mg, 3.00 mmol, 50%).

Rf = 0.57 (20% EtOAc in hexanes).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.51 – 7.42 (m, 2H), 4.08 (s, 2H), 1.56 (s, 9H).

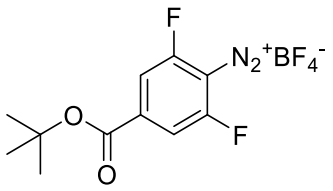
^{13}C NMR (126 MHz, $CDCl_3$) δ = 164.4, 150.8 (dd, *J* = 241, 8 Hz), 128.5 (t, *J* = 16 Hz), 120.5 (t, J = 8 Hz), 112.8 – 112.4 (m), 81.3, 28.3.

^{19}F NMR (377 MHz, $CDCl_3$) δ = -133.6.

IR: 3495, 3388, 2926, 1697, 1637, 1591, 1541, 1455, 1423, 1345, 1282, 1253, 1151, 964, 887, 765, 721, 560.

EI-HRMS: calcd. for $C_{11}H_{13}F_2NO_2$ $[M+H]^+$ 229.0909, found 229.0903.

4-(tert-butoxycarbonyl)-2,6-difluorobenzenediazonium tetrafluoroborate (11)



In a flame-dried flask, $NOBF_4$ (440 mg, 3.77 mmol, 1.5 equiv) was dissolved in anhydrous EtOAc (3.1 ml). The solution was cooled to 0 °C and subsequently, a solution of amine **10** (578 mg, 2.52 mmol, 1.00 equiv) in anhydrous EtOAc (3.1 ml, 0.4 M in total) was added dropwise but quickly to the reaction mixture which was left to stir at 0 °C for 1 hour. The reaction mixture was filtered through a glass filter and the residual solid was washed with ice-cold diethyl ether to afford 11 as a white crystalline powder (594 mg, 1.81 mmol, 72%).

^{1}H NMR (500 MHz, CD_3CN) δ = 8.16 – 8.05 (m, 2H), 1.61 (s, 9H).

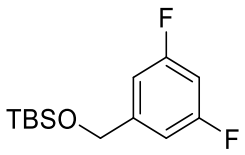
^{13}C NMR (126 MHz, CD_3CN) δ = 161.91 (d, J = 281 Hz), 161.0, 149.2, 116.56 – 116.26 (m), 99.4, 86.6, 28.0.

^{19}F NMR (471 MHz, CD_3CN) δ = -97.4, -151.8, -151.9.

IR: 2984, 2297, 1723, 1623, 1591, 1459, 1372, 1314, 1276, 1206, 1155, 1072, 889, 837, 772, 523.

ESI-HRMS: calcd. for $C_{11}H_{11}F_2N_2O_2^+$ $[M]^+$ 241.0783, found 241.0788.

***tert*-butyl((3,5-difluorobenzyl)oxy)dimethylsilane (12)**



(3,5-Difluorophenyl)methanol (2.00 g, 13.9 mmol, 1.00 equiv) was dissolved in anhydrous CH_2Cl_2 (55 ml, 0.25 M). Imidazole (1.89 g, 27.8 mmol, 2.00 equiv) and TBSCl (2.10 g, 13.9 mmol, 1.00 equiv) were added to the reaction mixture at room temperature and the resulting suspension was stirred for 16 hours. The reaction mixture was quenched by addition of sat. aq. NaHCO3 and the mixture was diluted with water and CH_2Cl_2. The phases were separated, and the combined org. layers were washed with H_2O (3x) and brine and dried over Na_2SO_4. After concentrating in vacuo, the crude product was further purified by flash column chromatography (30% EtOAC in hexanes) to afford **12** (3.51 g, 13.6 mmol, 98%) as a clear oil. Residual traces of TBSOH and TBSOTBS were removed on high vacuum.

Rf = 0.77 (20% EtOAc in hexanes).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.88 – 6.81 (m, 2H), 6.66 (tt, J = 9.0, 2.4 Hz, 1H), 4.71 (d, J = 1.0 Hz, 2H), 0.95 (s, 9H), 0.11 (s, 6H), 26.0, 18.5, -5.2.

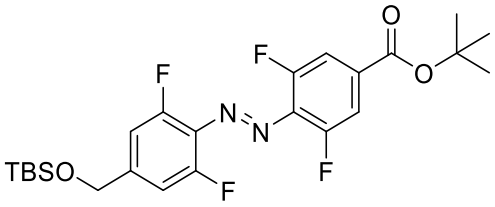
^{13}C NMR (101 MHz, $CDCl_3$) δ = 163.2 (dd, J = 248, 13 Hz), 145.9 (t, J = 9 Hz), 108.5 (dd, J = 19, 7 Hz), 102.2 (t, J = 26 Hz), 64.1 (t, J = 2 Hz).

^{19}F NMR (377 MHz, $CDCl_3$) δ = -110.8.

IR: 2956, 2931, 2859, 1627, 1596, 1460, 1373, 1323, 1258, 1143, 1116, 1100,964, 837, 777, 670537, 510.

EI-HRMS: calcd. for $C_9H_{11}OF_2Si$ [M – *tert*-Bu]$^+$ 201.05417, found 201.05401.

***tert*-butyl (E)-4-((4-(((tert-butyldimethylsilyl)oxy)methyl)-2,6-difluorophenyl)diazenyl)-3,5-difluorobenzoate (13)**



In a flame-dried flask, silyl ether **12** (446 mg, 1.73 mmol, 1.00 equiv) was dissolved in anhydrous THF (3.0 ml, 0.58 M). The solution was cooled to -78 °C and *t*-BuLi (1.7 M in pentane, 1.02 ml, 1.00 equiv) was added dropwise. The mixture was left to stir for one hour during which the mixture was allowed to warm up to -50 °C. Meanwhile, in another flame-dried flask diazonium salt **11** (566 mg, 1.73 mmol, 1.00 equiv) was suspended in anhydrous THF (3.0 ml, 0.58 M). Then, the lithiated mixture was cooled back to -78 °C and was quickly added to the diazonium salt suspension at -78°C by cannulation. Residual lithiated material was rinsed by anhydrous THF (2x 1.5 ml) followed by cannulation to the reaction mixture. The obtained dark red mixture was stirred for another hour during which it warmed to room temperature. The mixture was then quenched by addition of sat. aq. $NaHCO_3$ and diluted with EtOAc and H_2O. The phases were separated, and the aq. phase was extracted with EtOAc (3x). The combined org. layers were washed with brine and dried over Na_2SO_4. After concentrating under reduced pressure, the crude product was further purified by flash column chromatography (40% CH_2Cl_2 in hexanes) to afford **13** (650 mg, 1.30 mmol, 76%) as a red oil.

Rf = 0.69 (20% EtOAc in hexanes).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.49 – 7.43 (m, 1H), 6.85 – 6.80 (m, 1H), 4.66 (s, 1H), 1.56 (s, 5H), 0.92 (s, 5H), 0.08 (s, 3H).

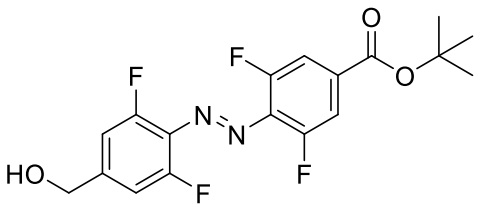
^{13}C NMR (101 MHz, $CDCl_3$) δ = 62.9 (t, J = 3.0 Hz), 151.8 (dd, J = 254, 5 Hz), 151.4 (dd, J = 254, 5 Hz), 146.0 (t, J = 8 Hz), 135.0, 133.7 (t, J = 8 Hz), 130.4, 113.6 – 113.2 (m), 109.4 – 108.8 (m), 82.9, 63.6 (t, J = 2 Hz), 28.16, 25.97, 18.46, -5.27.

^{19}F NMR (377 MHz, $CDCl_3$) δ = -119.0, -119.6.

IR: 2956, 2931, 2886, 1722, 1627, 1578, 1430, 1370, 1340, 125, 1159, 1045, 838, 770.

ESI-HRMS: calcd. for $C_{24}H_{30}F_4N_2NaO_3Si$ $[M+Na]^+$ 521.1854, found 521.1848.

***tert*-butyl (E)-4-((2,6-difluoro-4-(hydroxymethyl)phenyl)diazenyl)-3,5-difluorobenzoate (S3)**



Silyl ether **13** (815 mg, 1.64 mmol, 1.00 equiv) was dissolved in anhydrous THF (16.0 ml). While cooling to 0 °C, TBAF (1M in THF, 1.64 ml, 1.00 equiv) was added dropwise to the reaction mixture. After 15 minutes, the reaction mixture was quenched by addition of sat. aq. NH_4Cl and H_2O. The mixture was diluted with EtOAc and the phases were separated. The aq. phase was extracted with EtOAc (3x) and the combined org. layers were washed with brine and dried over Na_2SO_4. The crude product was further purified by flash column chromatography (40% EtOAc in hexanes) to afford **13** as a red crystalline solid (478 mg, 1.24 mmol, 76%).

Rf = 0.41 (40% EtOAc in hexanes).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.68 – 7.62 (m, 2H), 7.13 – 7.07 (m, 2H), 4.78 (d, J = 5.9 Hz, 2H), 1.93 (t, *J* = 5.9 Hz, 1H), 1.61 (s, 9H).

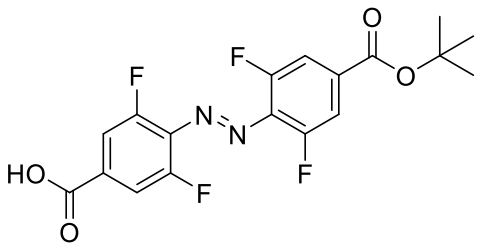
^{13}C NMR (101 MHz, $CDCl_3$) δ = 163.0 (t, J = 3 Hz), 156.1 (dd, J = 262, 4 Hz), 155.0 (dd, J = 262, 4 Hz), 147.2 (t, J = 9 Hz), 134.7 (t, J = 9 Hz), 134.4 (d, J = 10 Hz), 130.5 (t, J = 10 Hz), 113.8 (dd, J = 23.0, 3 Hz), 110.3 (dd, J = 21.2, 3 Hz), 82.9, 63.9 (t, J = 2 Hz), 28.2.

^{19}F NMR (377 MHz, $CDCl_3$) δ = -119.8, -121.2.

IR: 3439, 2984, 1718, 1627, 1582, 1478, 1438, 1368, 1335, 1250, 1156, 1039, 966, 885, 849, 769.

ESI-HRMS: calcd. for $C_{18}H_{16}F_4N_2NaO_3$ $[M+Na]^+$ 407.0989, found 407.0986.

(E)-4-((4-(tert-butoxycarbonyl)-2,6-difluorophenyl)diazenyl)-3,5-difluorobenzoic acid (14)



Alcohol **S3** (431 mg, 1.12 mmol, 1.00 equiv) was dissolved in a mixture of acetonitrile (7.5 ml) and an aq. pH 6.8 phosphate buffer (3.7 ml, 0.1 M in total). TEMPO (18.2 mg, 0.116 mmol, 10.0 mol%), sodium chlorite (80%, 256 mg, 2.26 mmol, 2.00 equiv) and aq. sodium hypochlorite (14%, 10.0 μl, 22.0 μmol, 2.00 mol%) were added to the reaction mixture. The reaction mixture was stirred for 3 hours at room temperature and was then quenched by addition of 5% aq. $Na_2S_2O_3$ and diluted with H_2O and brine. Phases were separated, and the aq. phase was extracted with EtOAc (3x). The combined organic layers were washed with 1M aq. HCl, brine and dried over Na_2SO_4. After concentration under reduced pressure, **14** was obtained as a red solid (450 mg, 1.12 mmol, quantitative yield) which was used without further purification.

Rf = 0.23 (10% MeOH in CH_2Cl_2).

^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 7.86 – 7.81 (m, 2H), 7.80 – 7.75 (m, 2H), 1.65 (s, 9H).

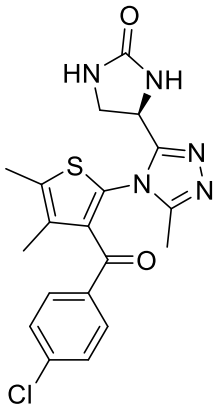
^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ = 165.7, 163.2, 157.1 (t, J = 4 Hz), 154.5 (t, J = 4 Hz), 137.3 (t, J = 9 Hz), 136.7 (t, J = 9 Hz), 134.5 (d, J = 10 Hz), 134.3 (d, J = 10.3 Hz), 114.6 (t, J = 23 Hz), 114.6 (t, J = 23 Hz), 83.5, 28.1.

^{19}F NMR (377 MHz, $(CD_3)_2CO$) δ = -120.3, -120.3.

IR: 2924, 2853, 1720, 1579, 1436, 1368, 1338, 1251, 1159, 1051, 770, 498.

ESI-HRMS: calcd. for $C_{18}H_{13}F_4N_2O_4$ $[M-H]^-$ 397.0817, found 397.0817.

(R)-4-(4-(3-(4-chlorobenzoyl)-4,5-dimethylthiophen-2-yl)-5-methyl-4H-1,2,4-triazol-3-yl)imidazolidin-2-one (16)



JQ-1 acid **15** (10.0 mg, 25.0 μmol, 1.00 equiv) was dissolved in a mixture of anhydrous toluene and freshly distilled *tert*-BuOH (4:1, 0.5 ml, 0.05 M). Et_3N (4.20 μl, 30.0 μmol, 1.20 equiv) and DPPA (5.40 μl, 25.0 μmol, 1.00 equiv) were added to the reaction mixture which was heated to reflux for 12 hours. The mixture was quenched by addition of sat. aq. $NaHCO_3$ and was further diluted with EtOAc and H_2O. The phases were separated, and the aq. phase was extracted with EtOAc and the combined org. layers were washed with brine and dried over Na_2SO_4. The crude product was further purified by column chromatography (10% MeOH in CH_2Cl_2) to furnish **16** as a yellow oil (6.2 mg, 15.0 μmol, 60%).

Rf = 0.19 (10% MeOH in CH_2Cl_2).

IR: 3238, 2924, 1709, 1658, 1586, 1487, 1402, 1267, 1234, 1008, 911, 731.

ESI-HRMS: calcd. for $C_{19}H_{19}ClN_5O_2$ $[M+H]^+$ 416.0942, found 416.0943.

16 was isolated as a 1.16:1 mixture of 2 atropisomers.

16-major

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.72 – 7.69 (m, 2H), 7.49 – 7.47 (m, 2H), 4.89 – 4.85 (m, 1H), 4.14 (t, J = 7.7 Hz, 1H), 3.74 (t, J = 8.9 Hz, 1H), 2.46 (d, J = 0.7 Hz, 3H), 2.17 (s, 3H), 1.95 – 1.94 (m, 3H).

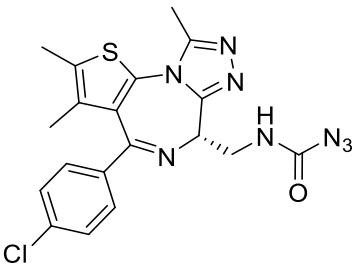
^{13}C NMR (126 MHz, $CDCl_3$) δ = 190.7, 162.4, 155.4, 153.7, 141.8, 141.1, 136.6, 134.2, 131.5, 131.2, 129.8, 127.6, 47.5, 44.8, 13.8, 13.5, 11.0.

16-minor

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.68 – 7.65 (m, 2H), 7.47 – 7.44 (m, 2H), 5.11 (t, J = 8.0 Hz, 1H), 4.08 (t, J = 8.0 Hz, 1H), 3.86 (t, J = 9.1 Hz, 1H), 2.46 (d, J = 0.7 Hz, 3H), 2.23 (s, 3H), 1.95 – 1.94 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 190.5, 162.3, 155.0, 154.2, 141.5, 140.3, 136.5, 134.5, 131.5, 130.9, 129.7, 127.9, 48.3, 44.9, 13.7, 13.6, 10.9.

JQ-1 azido carbamate ((S)-((4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)methyl)carbamoyl azide) (17)



JQ-1 acid **16** (17.2 mg, 43.0 μmol, 1.00 equiv) was dissolved in anhydrous THF (340 μl, 0.125 M). DIPEA (23.0 μl, 129 μmol, 3.00 equiv), 50% T3P in DMF (26.0 μl, 51.0 μmol, 1.20 equiv), $TMSN_3$ (14.0 μl, 107 μmol, 2.50 equiv), $Zn(OTf)_2$ (1.56 mg, 4.30 μmol, 10.0 mol%) and freshly distilled *tert*-butanol (86.0 μl, 900 μmol, 20.0 equiv) were added to the reaction mixture.

The reaction mixture was heated to 50 °C for 12 hours, then 50% T3P in DMF (6.50 μl, 12.8 μmol, 0.3 equiv) and $TMSN_3$ (2.80 μl, 21.4 μmol, 0.5 equiv) were readded. After another 2 hours the reaction was quenched by addition of sat. aq. $NaHCO_3$. The mixture was diluted with EtOAc and brine and the aqueous phase was extracted with EtOAc (3x). The combined organic layers were washed with brine and dried over sodium sulfate. After concentrating under reduced pressure, the crude product **17** was obtained as a yellow oil (19 mg, quantitative) which was used as is in the subsequent step.

Rf = 0.57 (10% MeOH in CH_2Cl_2).

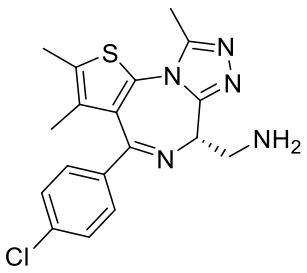
^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.44 (d, J = 8.6 Hz, 2H), 7.35 (d, J = 8.8 Hz, 2H), 6.30 – 6.23 (m, 1H), 4.35 – 4.29 (m, 3H), 2.68 (s, 3H), 2.41 (d, J = 0.8 Hz, 3H), 1.69 (d, J = 0.8 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 164.6, 157.2, 155.0, 150.2, 137.2, 136.5, 132.2, 131.2, 131.1, 130.5, 130.0, 128.9, 56.2, 42.3, 14.6, 13.3, 11.9.

IR: 3258, 2926, 2857, 2143, 1710, 1591, 1530, 1488, 1420, 1379, 1230, 1090, 1014, 841, 749.

ESI-HRMS: calcd. for $C_{19}H_{17}ClN_8NaOS$ $[M+Na]^+$ 463.0827, found 463.0826.

JQ-1 amine ((S)-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)methanamine) (18)



JQ-1 azido carbamate **17** (19.0 mg, 43.0 μmol, 1.00 equiv) was dissolved in anhydrous *tert*-Butanol (430 μl, 0.1 M). Deionized H_2O (1.60 μl, 86.0 μmol, 2.00 equiv) was added, followed by injecting 1 M KO*t*Bu in *tert*-Butanol (172 μl, 172 μmol, 4.00 equiv) directly into the solution at room temperature. The reaction mixture was stirred for 20 minutes and was then quenched by addition of sat. aq. $NaHCO_3$. The mixture was diluted with water, brine and EtOAc. The phases were separated, and the aq. phase was extracted with EtOAc (3x). The combined org layers were washed with brine, dried over sodium sulfate and filtered through a pad of Celite. After concentrating under reduced pressure, the crude product **18** was obtained as a yellow oil (15.0 mg, 40.0 μmol, 94% yield over 2 steps) and was submitted to the subsequent step without further purification.

Rf = 0.30 (20% MeOH in CH_2Cl_2).

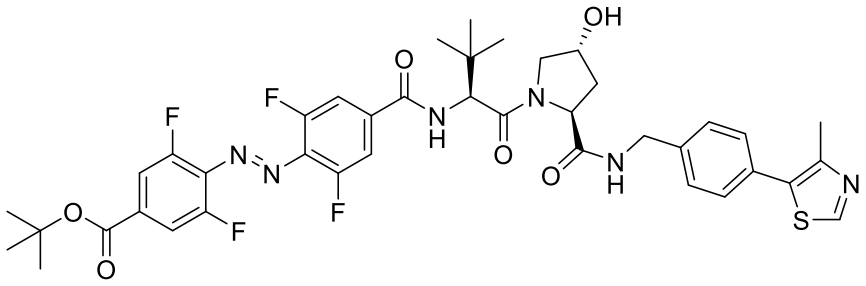
^{1}H NMR (500 MHz, CD_3OD) δ = 7.54 (d, J = 8.7 Hz, 2H), 7.42 (d, *J* = 8.7 Hz, 2H), 4.22 (dd, J = 7.2, 5.7 Hz, 1H), 3.70 (dd, J = 13.1, 7.3 Hz, 1H), 3.65 – 3.58 (m, 2H), 2.70 (s, 3H), 2.44 (d, J = 0.7 Hz, 3H), 1.70 (d, J = 0.7 Hz, 3H).

^{13}C NMR (126 MHz, CD_3OD) δ = 166.9, 156.3, 151.9, 138.2, 138.0, 133.5, 133.2, 132.0, 132.0, 131.4, 129.8, 59.2, 43.4, 14.4, 12.9, 11.6.

IR: 2924, 2855, 1591, 1555, 1531, 1488, 1421, 1398, 1379, 1314, 1262, 1314, 1262, 1090, 1014, 912, 840, 802, 731, 484.

ESI-HRMS: calcd. for $C_{18}H_{18}ClN_5S$ $[M+H]^+$ 372.1044, found 372.1040.

tert-butyl 4-((E)-(2,6-difluoro-4-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrolidine-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)phenyl)diazenyl)-3,5-difluorobenzoate (20)



19 (13.7 mg, 29.0 μmol, 1.00 equiv) and **14** (acid) (11.7 mg, 29.0 μmol, 1.00 equiv) were dissolved in anhydrous DMF (0.29 ml, 0.1 M). DIPEA (12.3 μl, 88.0 μmol, 3.00 equiv) and HATU (11.7 mg, 31.0 μmol, 1.05 equiv) were added to the reaction mixture at room temperature. After 2 hours, the reaction mixture was quenched by addition of sat. aq. $NaHCO_3$ and the aq. phase was extracted three times with EtOAc. The combined org. layers were washed with brine and dried over sodium sulfate. The crude product was further purified by flash column chromatography (5% MeOH in CH_2Cl_2) to afford **20** as an orange oil (21.0 mg, 26.0 μmol, 88%), still containing a tetramethyl urea impurity.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.71 (s, 1H), 7.68 – 7.64 (m, 2H), 7.48 – 7.44 (m, 2H), 7.39 – 7.34 (m, 4H), 7.18 (t, *J* = 6.0 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 1H), 4.74 (d, J = 8.5 Hz, 2H), 4.63 – 4.60 (m, 1H), 4.58 (dd, J = 15.0, 6.5 Hz, 1H), 4.35 (dd, J = 15.0, 5.2 Hz, 1H), 4.04 (dt, J = 11.5, 1.9 Hz, 1H), 3.72 (dd, J = 11.5, 3.9 Hz, 1H), 2.58 –2.53 (m, 1H), 2.52 (s, 3H), 2.17 – 2.11 (m, 1H), 1.61 (s, 9H), 1.02 (s, 9H).

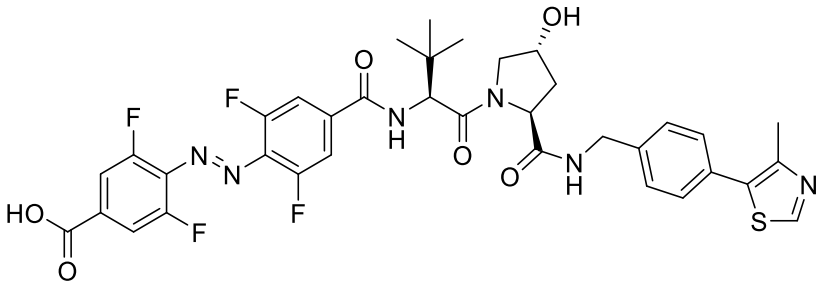
^{13}C NMR (126 MHz, $CDCl_3$) δ = 171.3, 170.7, 164.4, 162.8, 155.4 (dd, J = 263, 4 Hz), 155.1 (dd, J = 263, 4 Hz), 150.6, 148.4, 138.2, 137.1 (t, J = 8.5 Hz), 135.6 (t, J = 9.0 Hz), 134.0 (t, J = 10.0 Hz), 133.5 (t, J = 10.0 Hz), 131.9, 131.1, 129.7, 128.3, 113.9 (dd, J = 22, 4 Hz), 111.9 (dd, J = 22, 4 Hz), 83.1, 70.4, 58.9, 58.3, 57.0, 43.5, 36.2, 36.0, 28.2, 26.6, 16.1.

^{19}F NMR (471 MHz, $CDCl_3$) δ = -118.3, -119.7.

IR: 3315, 3073, 2960, 2930, 2874, 1721, 1667, 1626, 1522, 1431, 1370, 1341, 1251, 1159, 1049, 966, 887, 848, 770, 732.

ESI-HRMS: calcd. for $C_{40}H_{43}F_4N_6O_6S$ $[M+H]^+$ 811.2895, found 811.2885.

4-((E)-(2,6-difluoro-4-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)phenyl)diazenyl)-3,5-difluorobenzoic acid (S4)



Tert-butyl ester **20** (20.0 mg, 25.0 μmol, 1.00 equiv) was dissolved in CH_2Cl_2 (330 μl, 0.075 M) and TFA (160 μl) was added to the mixture at room temperature. After stirring for 1 hour, the solvents were removed under a constant vstream of N_2 and the resulting residue was dissolved in EtOAc and sat. aq. $NaHCO_3$. The mixture was diluted with water and brine and the phases were separated. The aq. phase was extracted with EtOAc (3x) and the combined org. layers were washed with brine and dried over Na_2SO_4. After concentration, the **S4** was obtained as a red oil (18.5 mg, 25.0 μmol, quantitative) and was used as is in the subsequent step.

Rf = 0.15 (20% MeOH in CH_2Cl_2).

^{1}H NMR (500 MHz, CD_3OD) δ = 9.11 (s, 1H), 7.82 – 7.75 (m, 2H), 7.73 – 7.67 (m, 2H), 7.53 – 7.43 (m, 4H), 4.91 (s, 1H), 4.65 – 4.60 (m, 1H), 4.59 (d, *J* = 15.5 Hz, 1H), 4.55 – 4.52 (m, 1H), 4.36 (d, J = 15.5 Hz, 1H), 3.99 (d, *J* = 11.1 Hz, 1H), 3.87 (dd, J = 11.1, 3.8 Hz, 1H), 2.50 (s, 3H), 2.32 – 2.22 (m, 1H), 2.12 (ddd, *J* = 13.3, 9.2, 4.3 Hz, 1H), 1.13 (s, 9H).

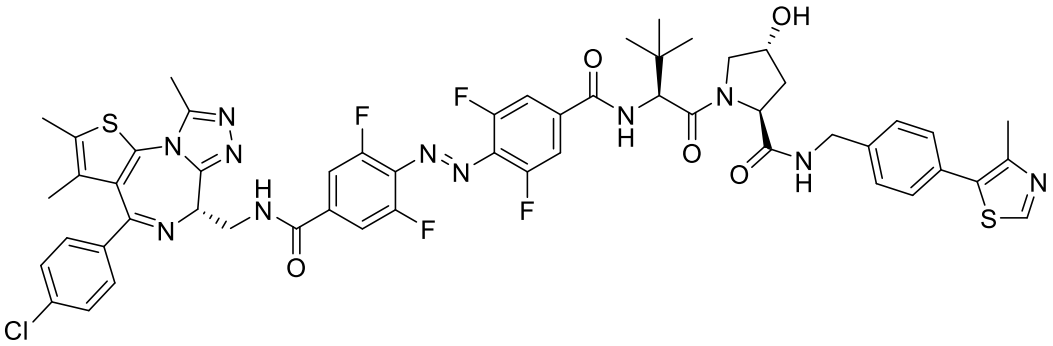
^{13}C NMR (126 MHz, CD_3OD) δ = 173.0, 170.6, 165.1 (t, *J* = 2.5 Hz), 164.9, 155.0 (dd, J = 261, 4 Hz), 154.8 (dd, *J* = 261, 4 Hz), 152.3, 146.4, 139.4, 137.9 (t, *J* = 9 Hz), 135.4, 134.6 (t, *J* = 9 Hz), 133.8, 132.9, 129.4, 129.0, 127.7, 113.6 (d, *J* = 25.5 Hz), 112.0 (d, *J* = 22.7 Hz), 69.7, 59.6, 58.6, 56.8, 42.3, 37.6, 35.8, 25.7, 13.9.

^{19}F NMR (471 MHz, CD_3OD) δ = -121.4, -122.1.

IR: 3321, 3084, 2958, 2926, 2855, 1712, 1666, 1627, 1578, 1532. 1434, 1325, 1196, 1052.

ESI-HRMS: calcd. for $C_{36}H_{34}F_4N_6O_6S$ $[M+H]^+$ 755.2269, found 755.2269.

(2S,4R)-1-((S)-2-(4-((E)-(4-((((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)methyl)carbamoyl)-2,6-difluorophenyl)diazenyl)-3,5-difluorobenzamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (photoPROTAC-1)



JQ-1 amine **18** (10.5 mg, 28.0 μmol, 1.00 equiv) and acid **S4** (21.4 mg, 28.0 μmol, 1.00 equiv) were dissolved in anhydrous DMF (0.28 ml, 0.1 M). DIPEA (12 μl, 85 μmol, 3.00 equiv) and HATU (11.3 mg, 30.0 μmol, 1.05 equiv) were added to the reaction mixture at room temperature. After 2 hours, the reaction mixture was quenched by addition of sat. aq. $NaHCO_3$ and the aq. phase was extracted three times with EtOAc. The combined org. layers were washed with brine and dried over sodium sulfate. Residual DMF and tetramethylurea were removed by lyophilization after freezing in a water/dioxane mixture. The crude product was further purified by flash column chromatography (94% EtOAc/4% iPrOH/2% H_2O) to afford **photoPROTAC-1** as an orange oil (16.0 mg, 14.0 μmol, 51%).

Rf = 0.36 (85% EtOAc/10% iPrOH/5% H_2O).

^{1}H NMR (500 MHz, CD_3OD) δ = 8.87 (s, 1H), 7.70 (dd, J = 5.1, 1.6 Hz, 2H), 7.67 (dd, J = 5.1, 1.6 Hz, 2H), 7.52 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H), 7.44 – 7.40 (m, 4H), 4.91 (s, 1H), 4.65 – 4.50 (m, 4H), 4.43 (dd, J = 13.6, 7.0 Hz, 2H), 4.35 (d, J = 15.4 Hz, 1H), 3.98 (d, J = 11.0 Hz, 1H), 3.87 (dd, J = 11.0, 3.8 Hz, 1H), 2.71 (s, 3H), 2.47 (s, 3H), 2.43 (s, 3H), 2.29 – 2.22 (m, 1H), 2.15 – 2.09 (m, 1H), 1.69 (s, 3H), 1.13 (s, 9H).

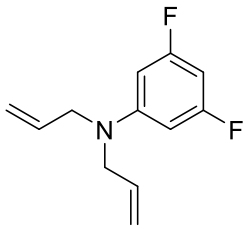
^{13}C NMR (126 MHz, CD_3OD) δ = 174.4, 172.0, 166.8, 166.7, 166.5, 157.4, 156.1, 155.3, 153.0, 152.2, 149.0, 140.3, 139.2, 138.1, 138.1, 134.3, 133.5, 133.4, 133.3, 133.3, 132.0, 132.0, 131.5, 131.4, 131.3, 130.4, 129.8, 129.0, 113.4, 113.1, 71.1, 60.9, 59.9, 58.2, 56.8, 43.7, 42.9, 39.0, 37.2, 27.1, 15.8, 14.4, 12.9, 11.6.

19F NMR (471 MHz, CD_3OD) δ = -121.4, -121.5.

IR: 3322, 2925. 28855, 1665, 1533, 1427, 1343, 1243, 1090, 1047, 967, 843.

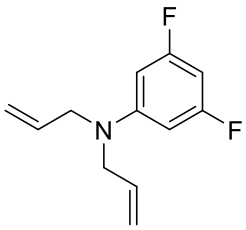
ESI-HRMS: calcd. for $C_{54}H_{51}ClF_4N_{11}O_5S_2$ $[M+H]^+$ 1108.3135, found 1108.3144.

4. NMR Spectra



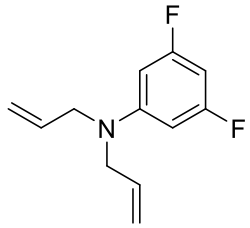
5

^{1}H-NMR ($CDCl_3$)



5

^{13}C-NMR ($CDCl_3$)



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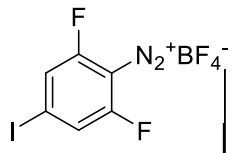
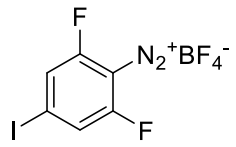
^{19}F-NMR ($CDCl_3$)



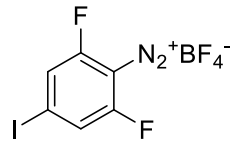
4

^{1}H-NMR (CD_3CN)



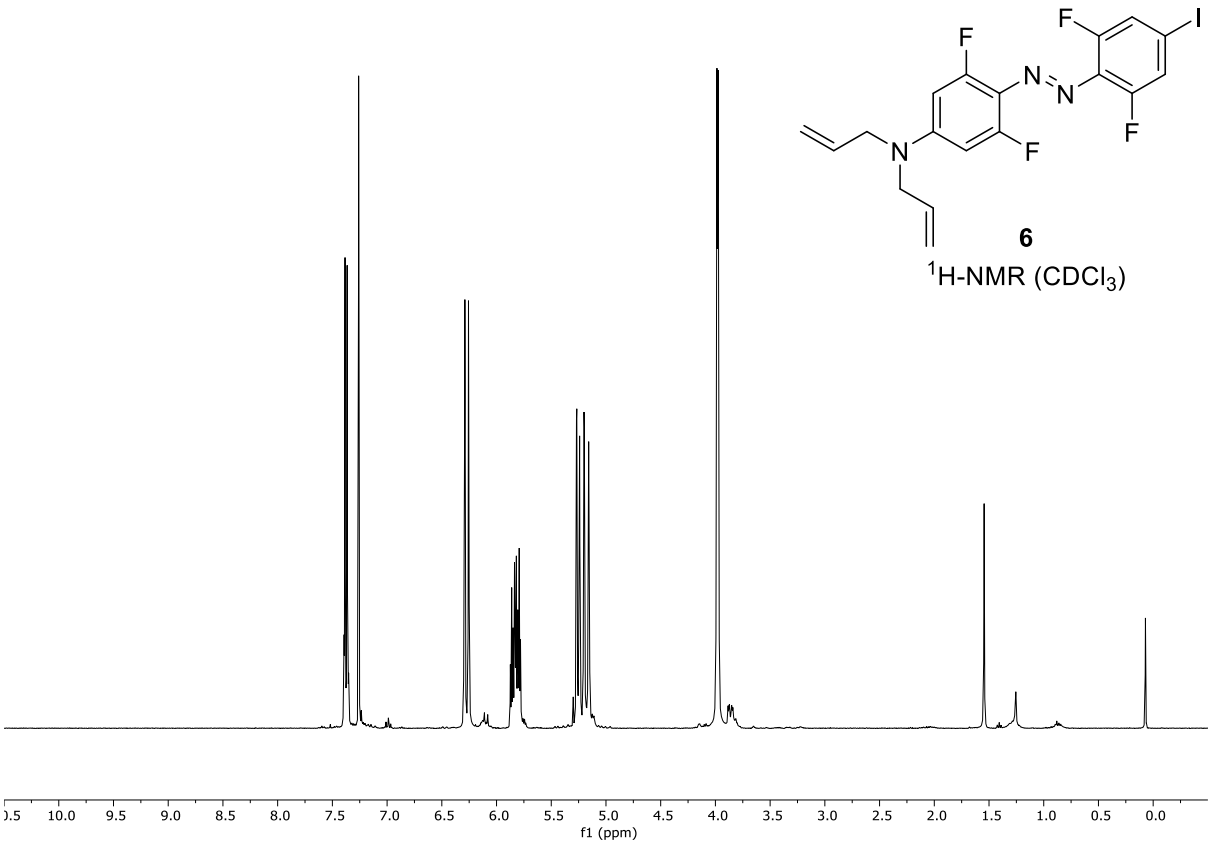
4

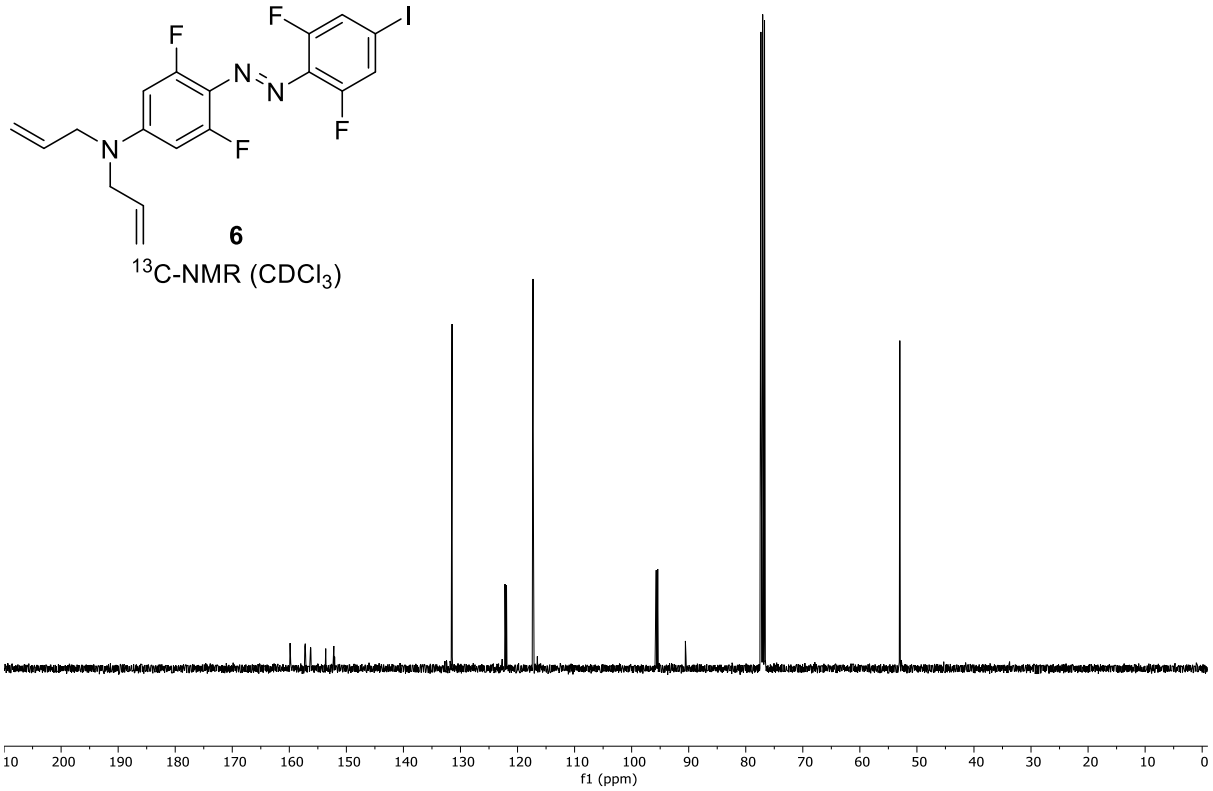
^{13}C-NMR (CD_3CN)

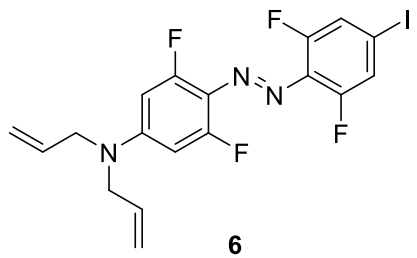


4

^{19}F-NMR (CD_3CN)

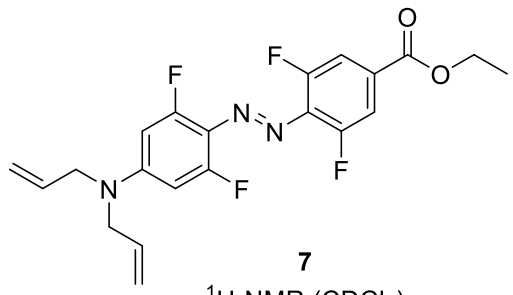

F
F
I
N
N
F
F
N
6
^{1}H-NMR ($CDCl_3$)
f1 (ppm)


F
F
I
N
N
F
F
N
6
^{13}C-NMR ($CDCl_3$)
f1 (ppm)



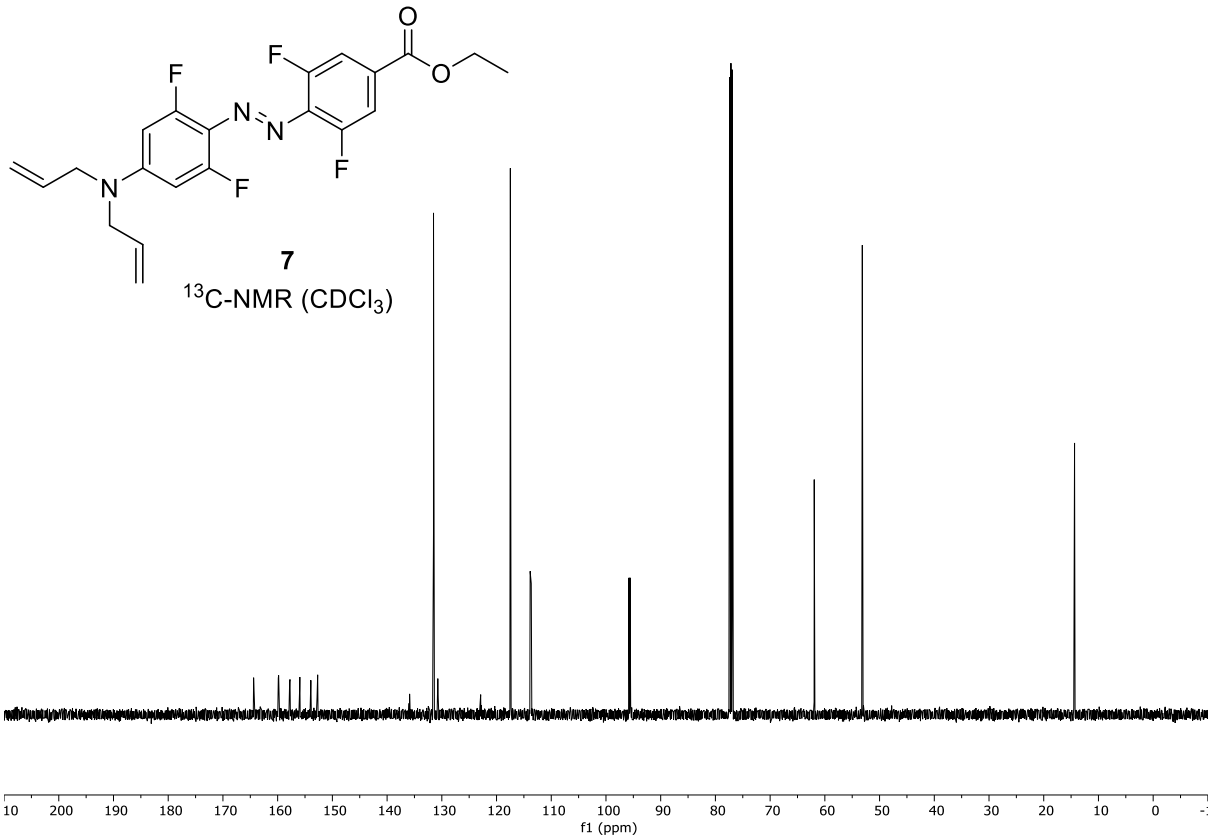
6

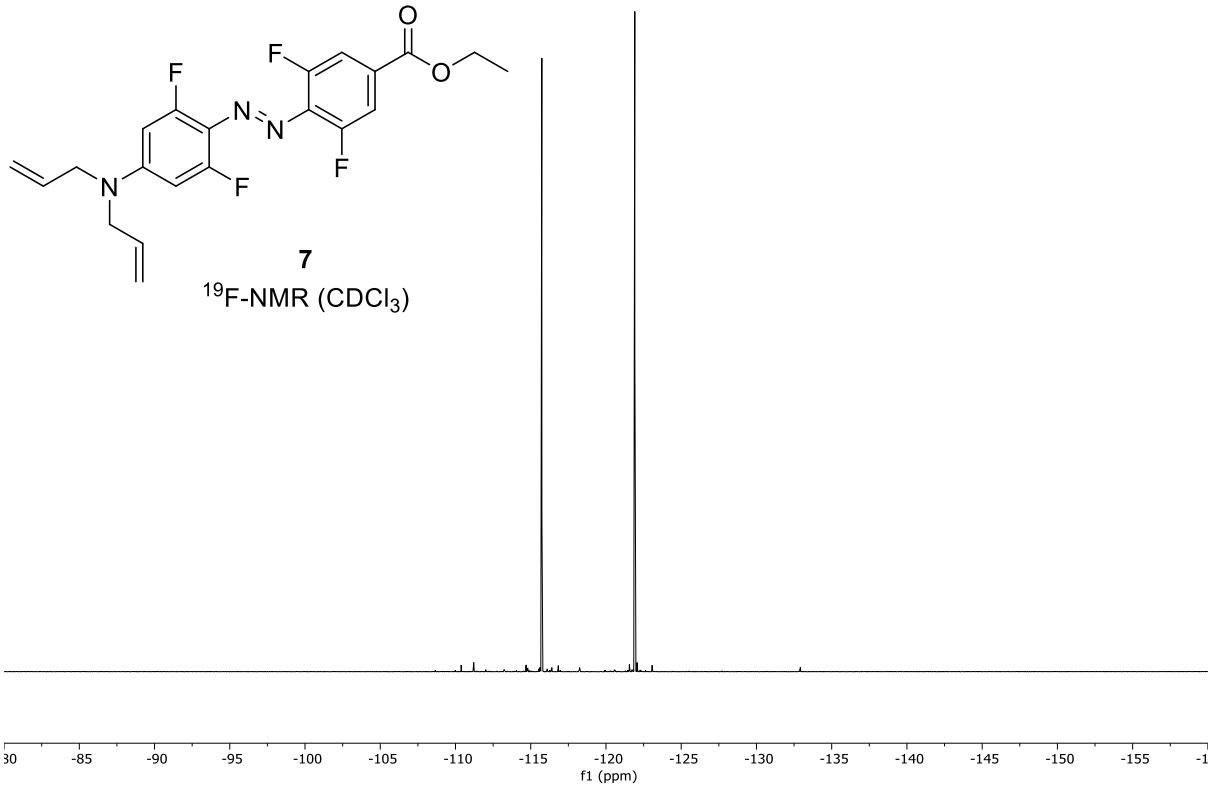
^{19}F-NMR ($CDCl_3$)

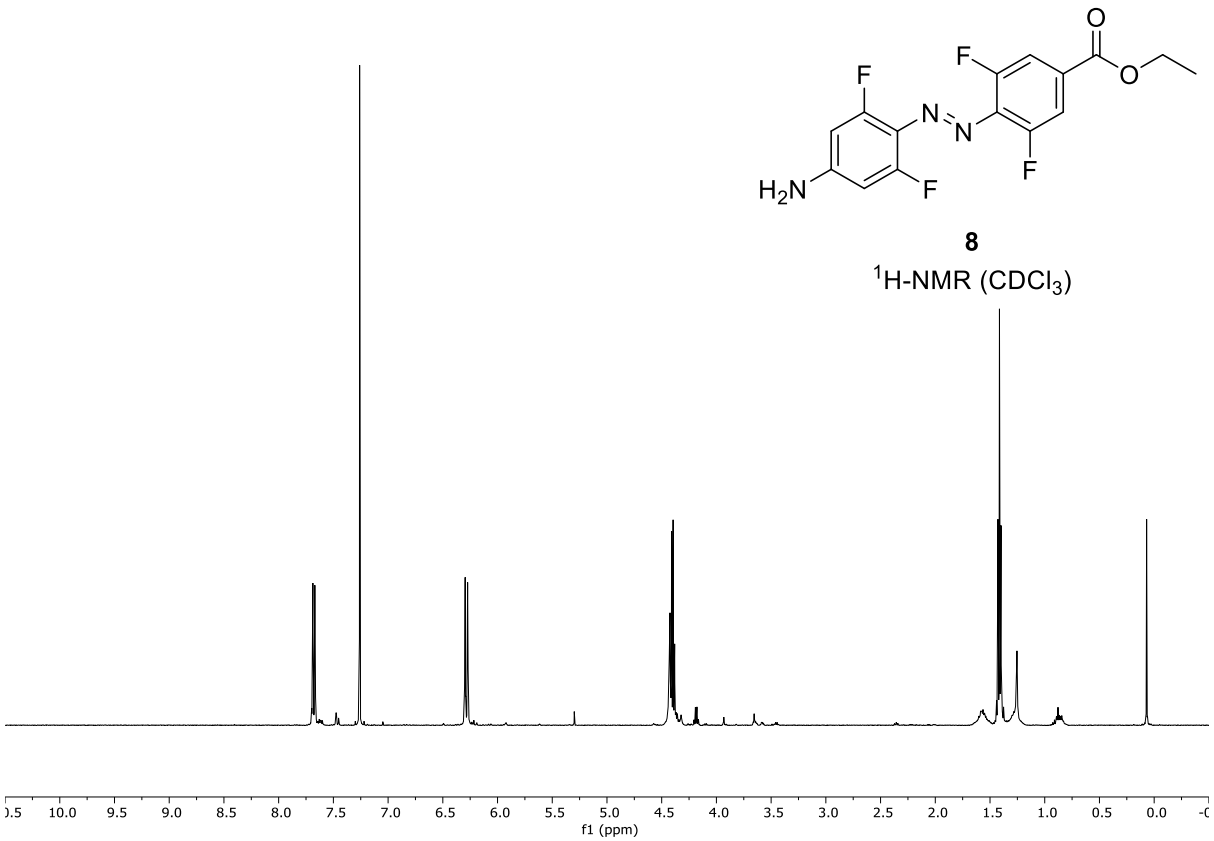


7

^{1}H-NMR ($CDCl_3$)

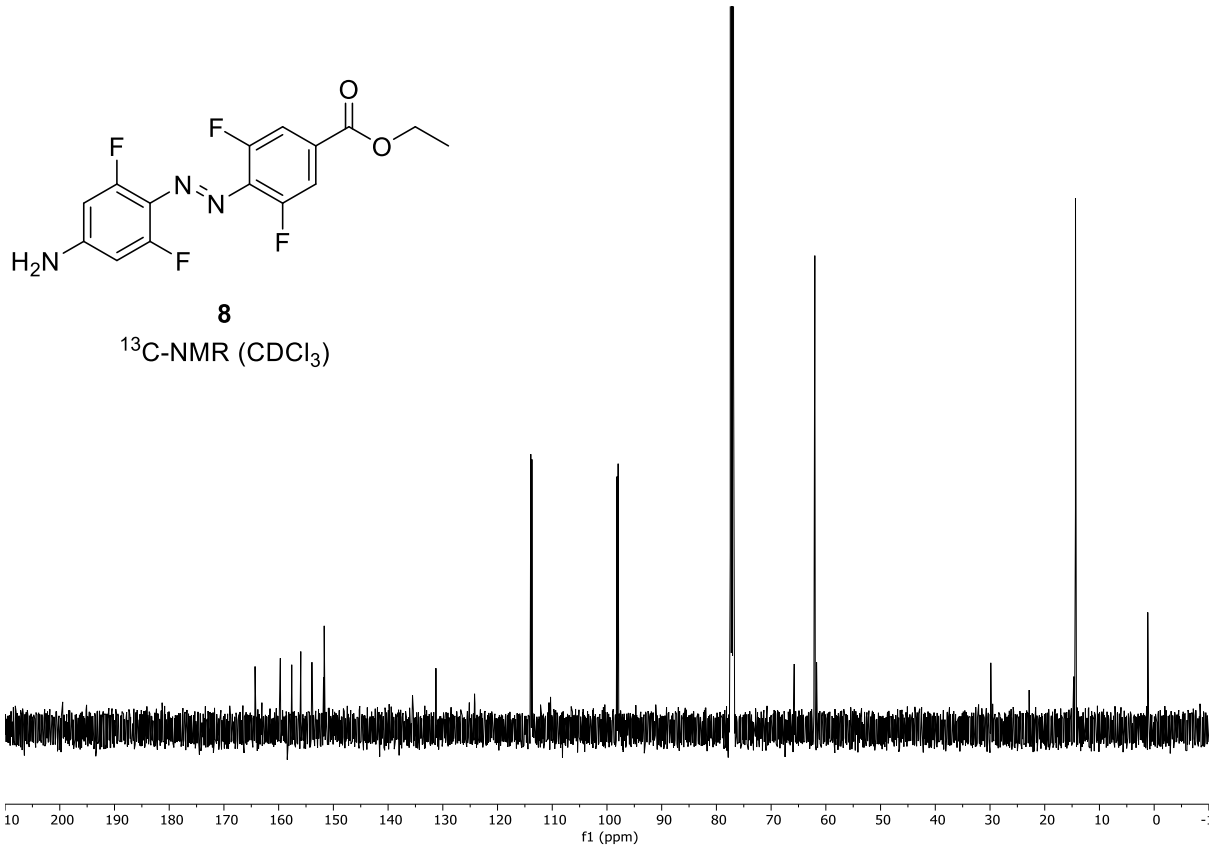

O
F
F
O
N
N
F
F
N
7
^{13}C-NMR ($CDCl_3$)
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
F
F
O
N
N
F
F
N
7
^{19}F-NMR ($CDCl_3$)
-85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145 -150 -155
f1 (ppm)



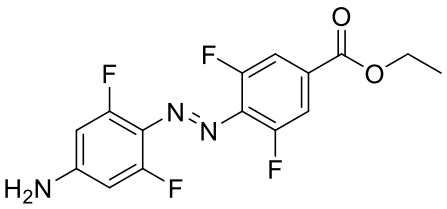
8

^{1}H-NMR ($CDCl_3$)



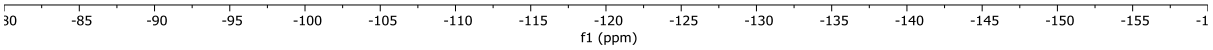
8

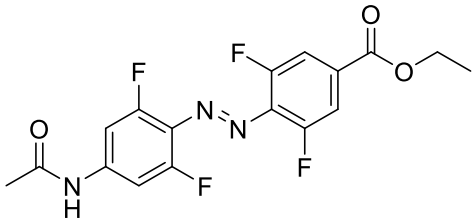
^{13}C-NMR ($CDCl_3$)



8

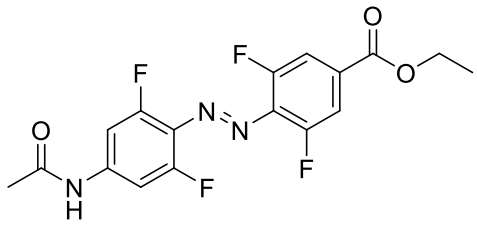
^{19}F-NMR ($CDCl_3$)





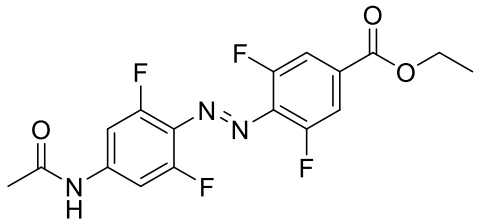
9

^{1}H-NMR ($CDCl_3$)



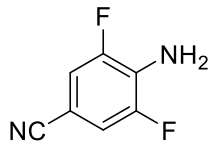
9

^{13}C-NMR ($CDCl_3$)



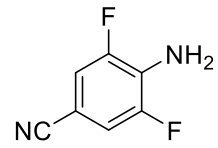
9

^{19}F-NMR ($CDCl_3$)



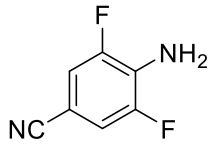
S1

^{1}H-NMR ($CDCl_3$)



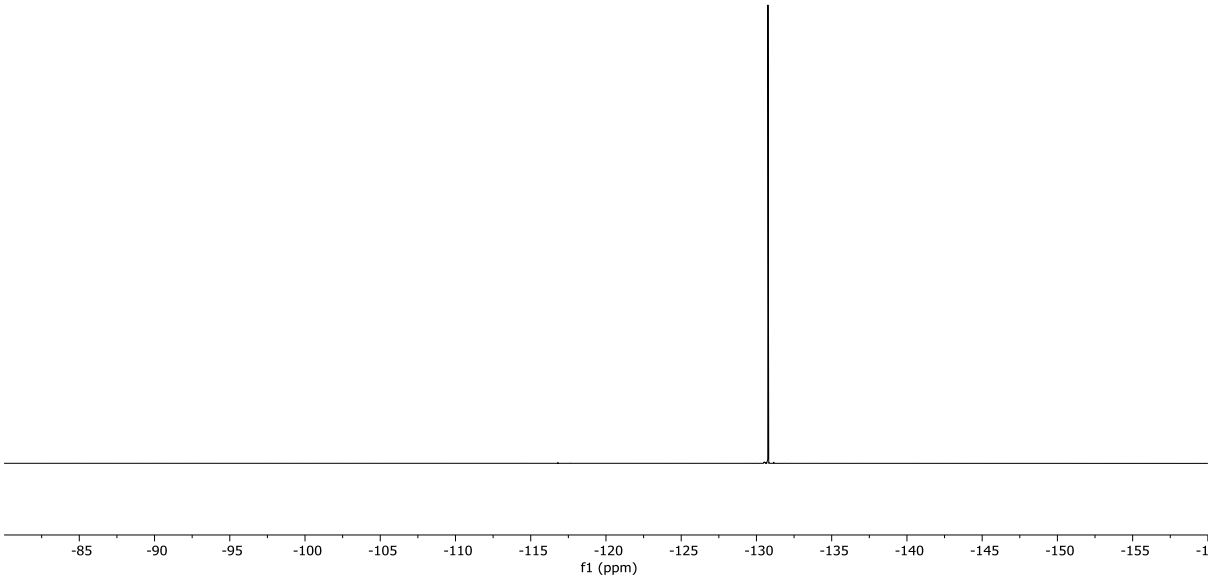
S1

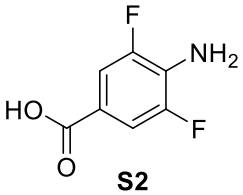
^{13}C-NMR ($CDCl_3$)



S1

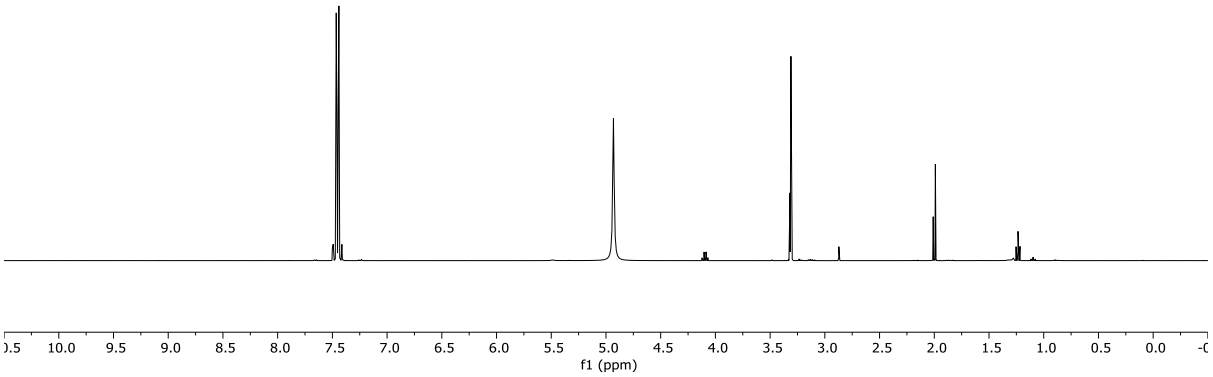
^{19}F-NMR ($CDCl_3$)

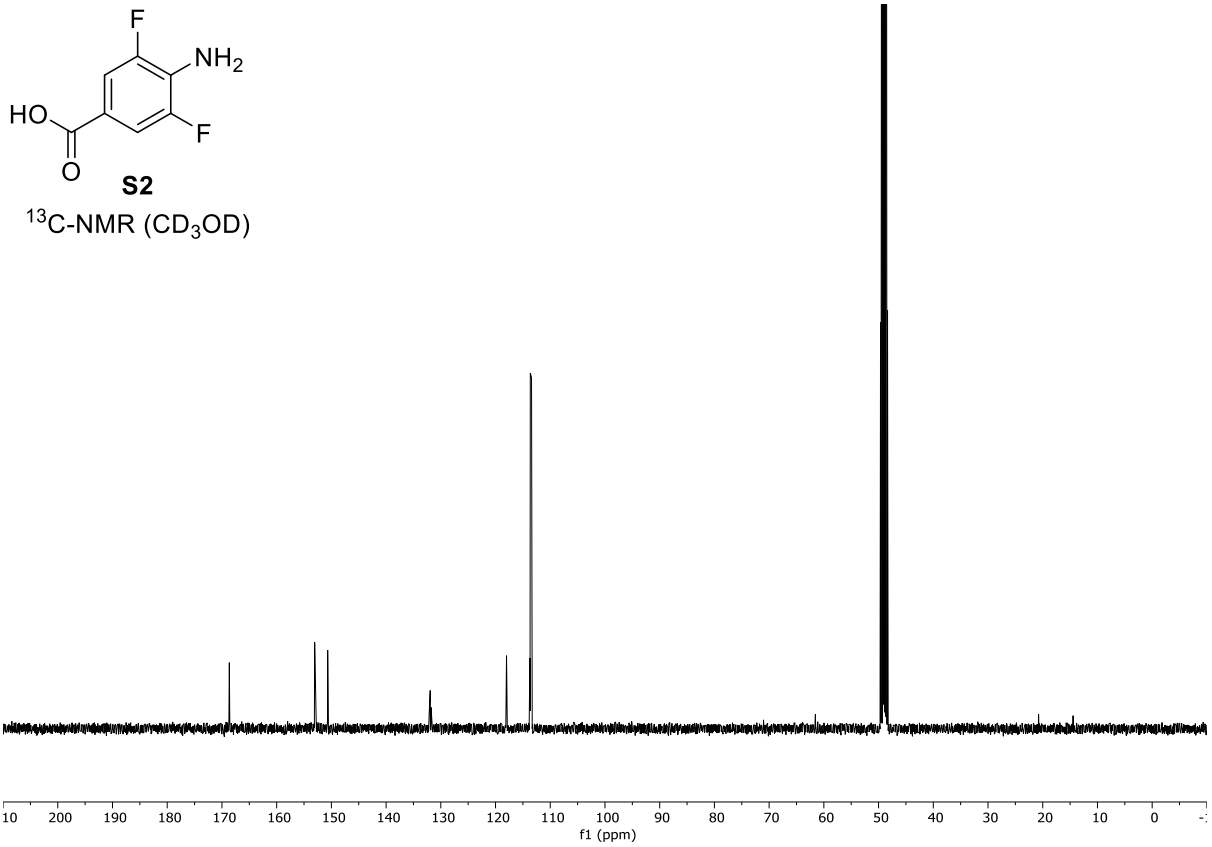


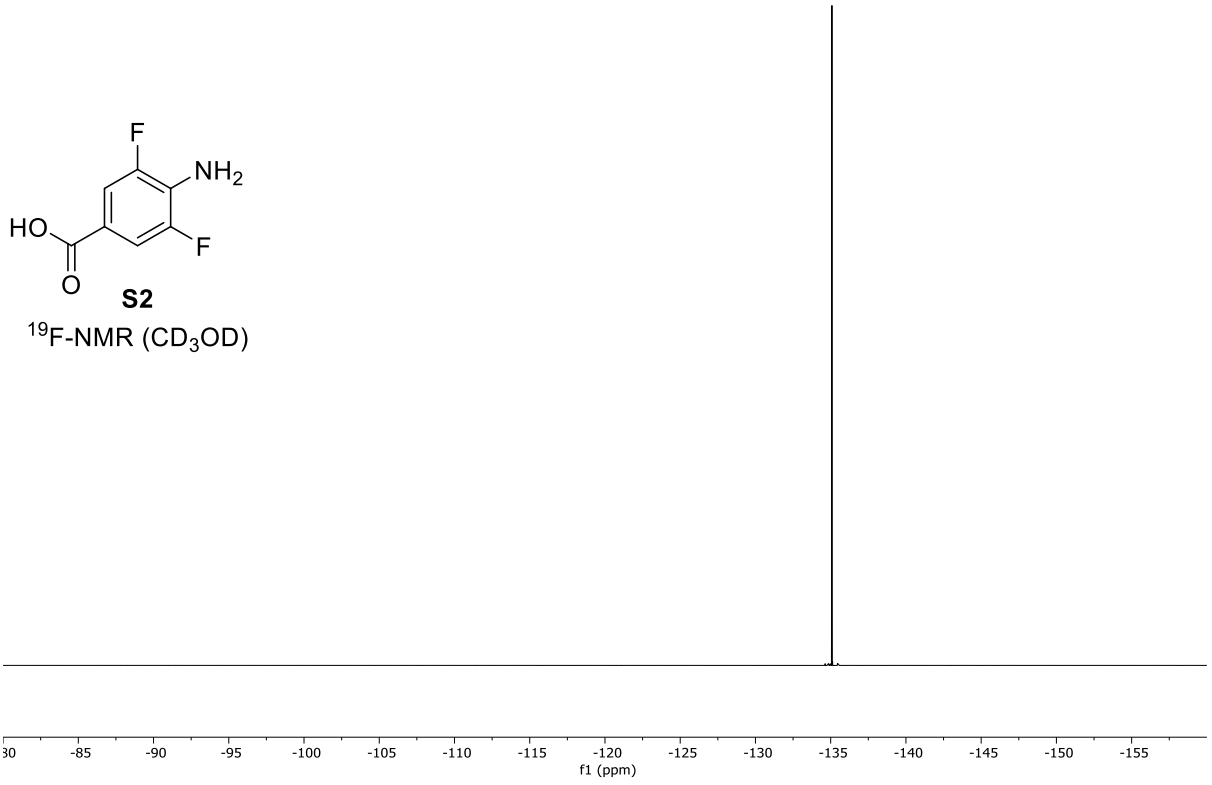


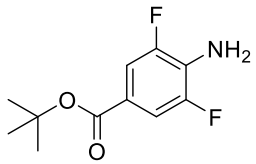
S2

^{1}H-NMR (CD_3OD)



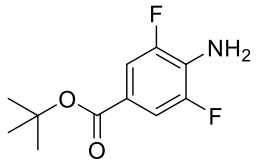

F
NH2
HO
F
O
S2
13C-NMR (CD3OD)
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


F
NH2
HO
F
O
S2
19F-NMR (CD3OD)
-85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145 -150 -155
f1 (ppm)



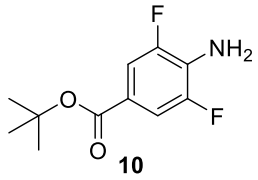
10

^{1}H-NMR ($CDCl_3$)



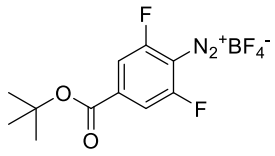
10

^{13}C-NMR ($CDCl_3$)



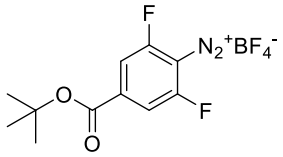
10

^{19}F-NMR ($CDCl_3$)



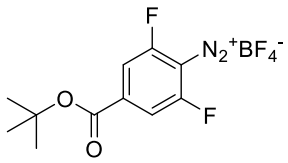
11

^{1}H-NMR (CD_3CN)



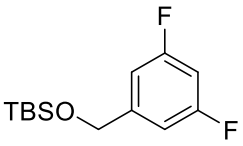
11

^{13}C-NMR (CD_3CN)



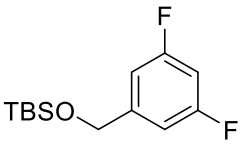
11

^{19}F-NMR (CD_3CN)



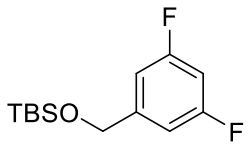
12

^{1}H-NMR ($CDCl_3$)



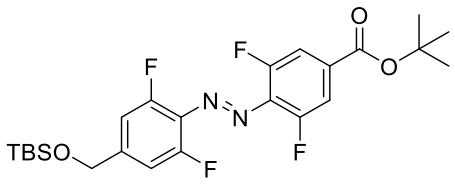
12

^{13}C-NMR ($CDCl_3$)



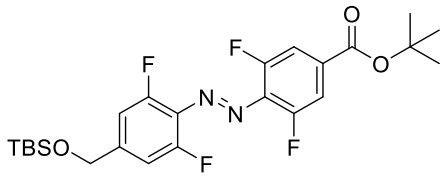
12

^{19}F-NMR ($CDCl_3$)



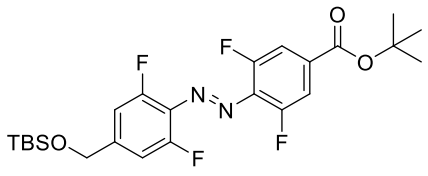
13

^{1}H-NMR ($CDCl_3$)



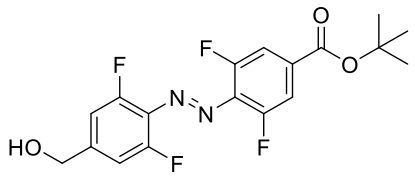
13

^{13}C-NMR ($CDCl_3$)



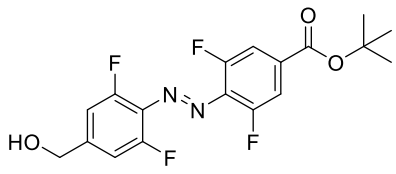
13

^{19}F-NMR ($CDCl_3$)



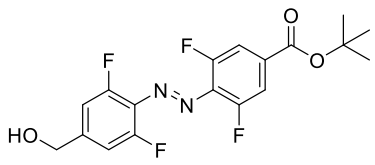
S3

^{1}H-NMR ($CDCl_3$)



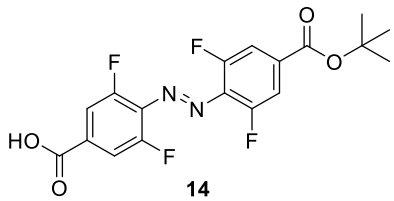
S3

^{13}C-NMR ($CDCl_3$)



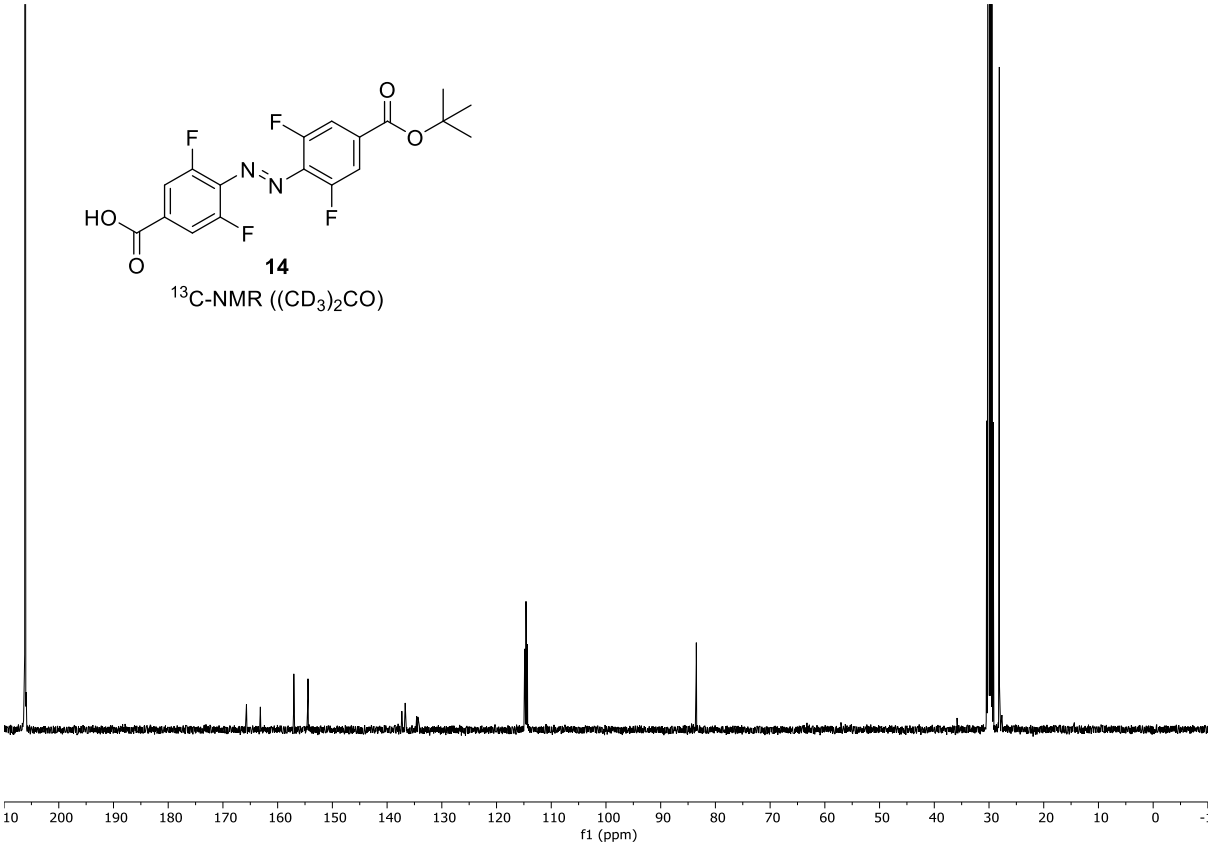
S3

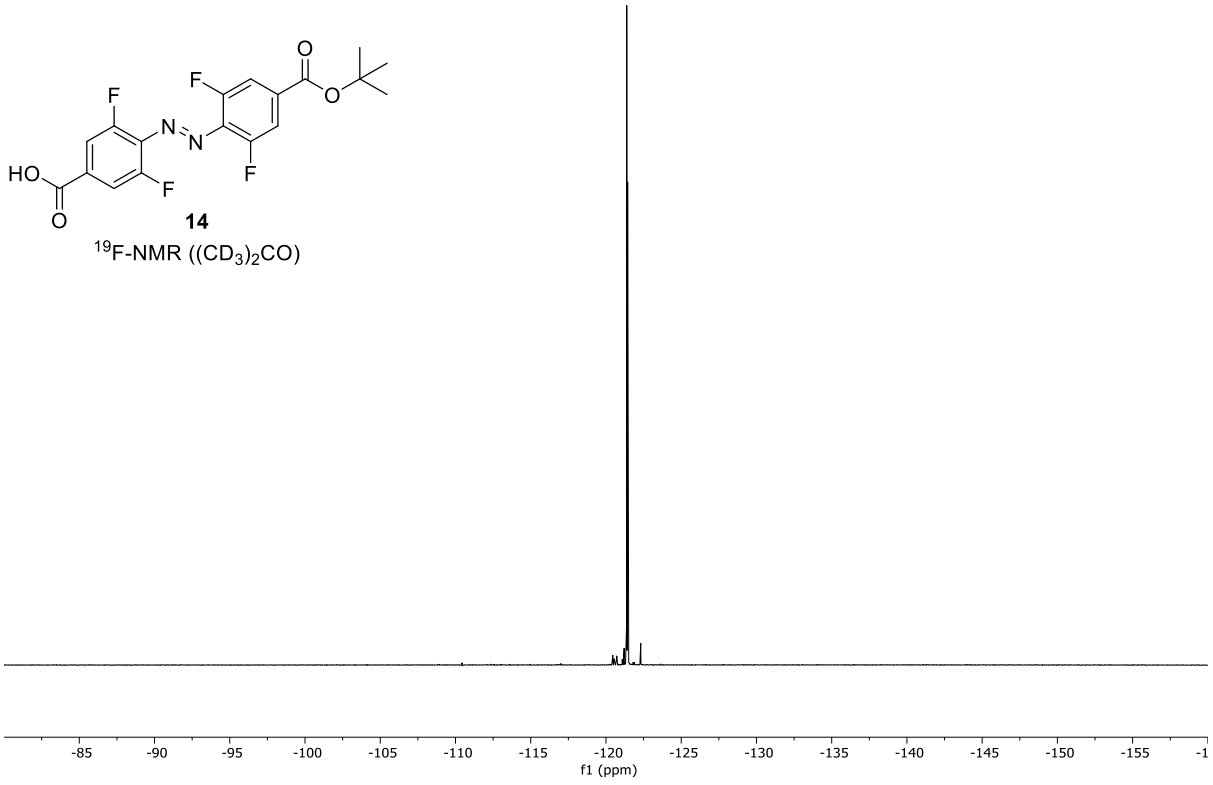
^{19}F-NMR ($CDCl_3$)

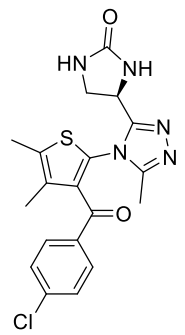


14

^{1}H-NMR ($(CD_3)_2CO$)

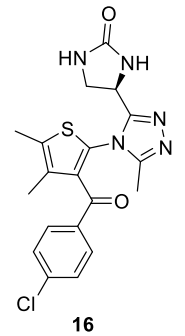

F
N
N
F
O
O
HO
F
F
O
14
13C-NMR ((CD3)2CO)
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


F
N
N
F
O
O
HO
F
F
O
14
19F-NMR ((CD3)2CO)
-85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145 -150 -155
f1 (ppm)



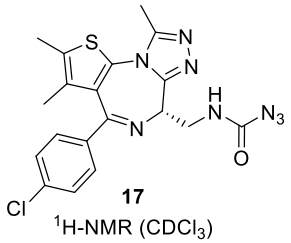
16

^{1}H-NMR ($CDCl_3$)



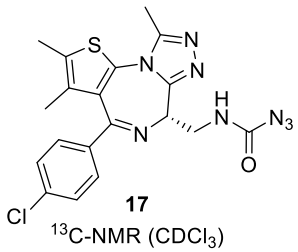
16

^{13}C-NMR ($CDCl_3$)



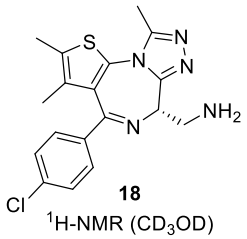
17

^{1}H-NMR ($CDCl_3$)



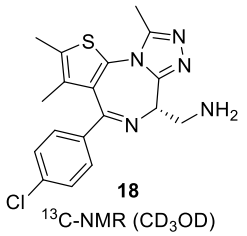
17

^{13}C-NMR ($CDCl_3$)



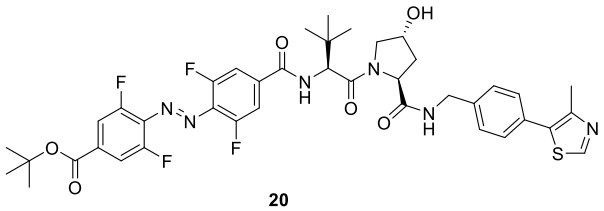
18

^{1}H-NMR (CD_3OD)



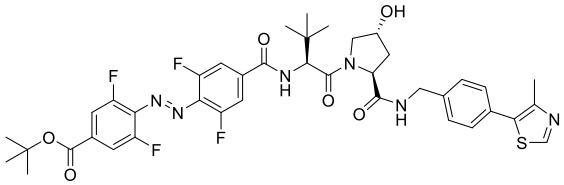
18

^{13}C-NMR (CD_3OD)



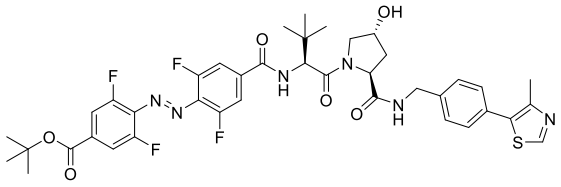
20

^{1}H-NMR ($CDCl_3$)



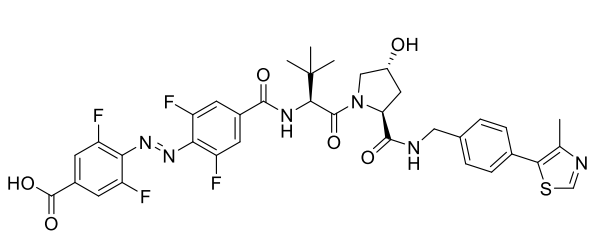
20

^{13}C-NMR ($CDCl_3$)



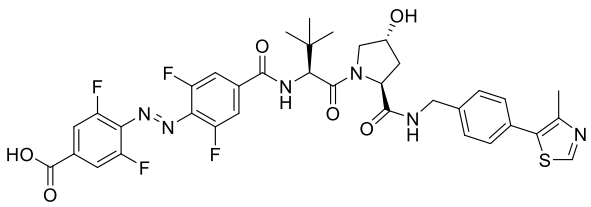
20

^{19}F-NMR ($CDCl_3$)



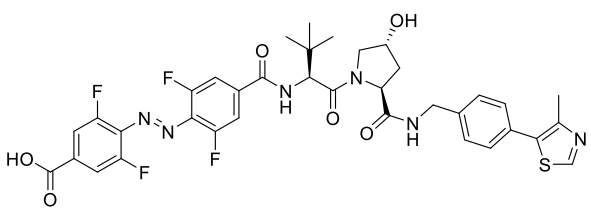
S4

^{1}H-NMR (CD_3OD)



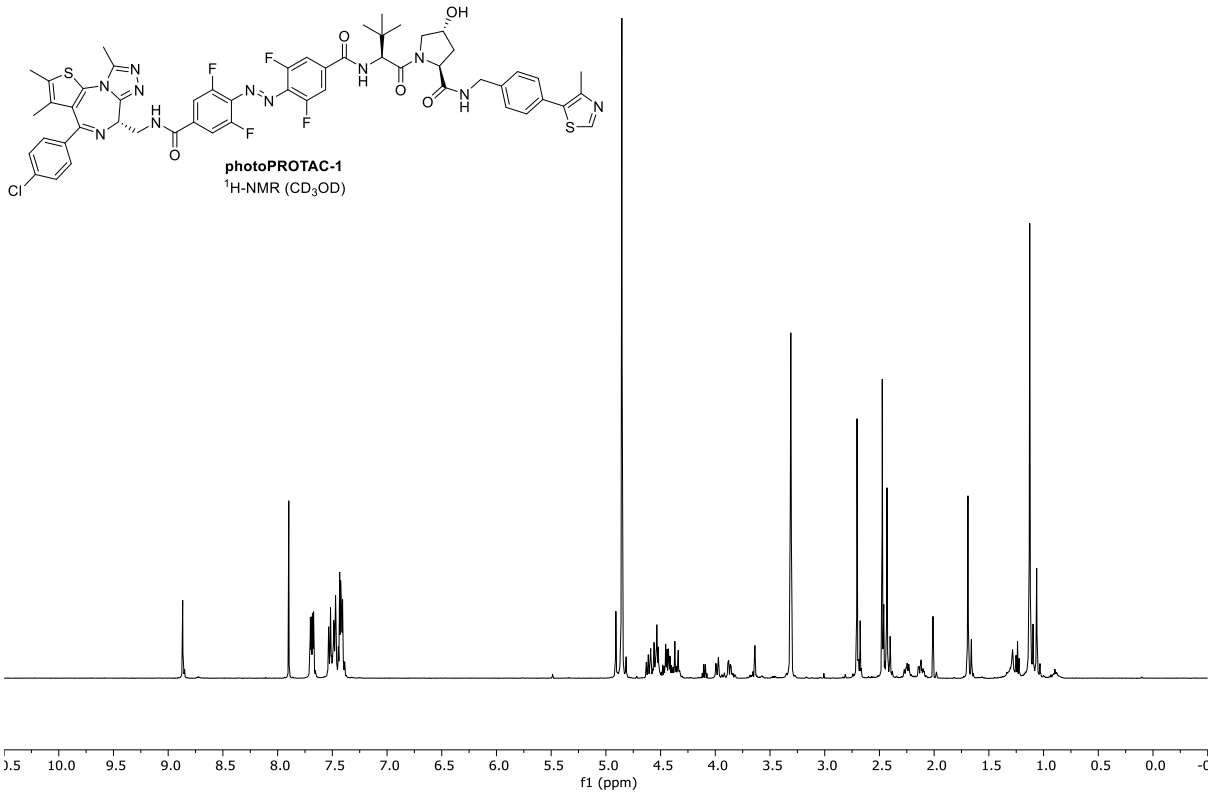
S4

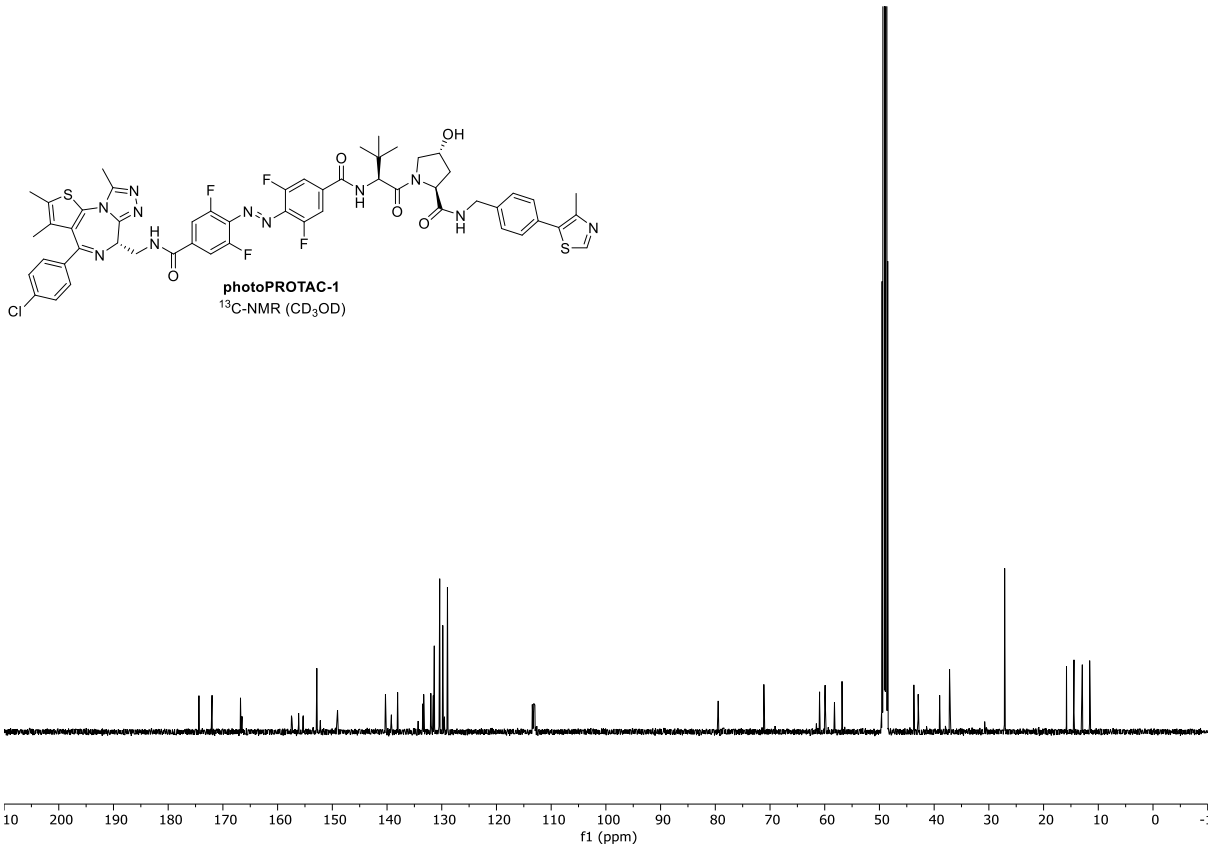
^{13}C-NMR (CD_3OD)

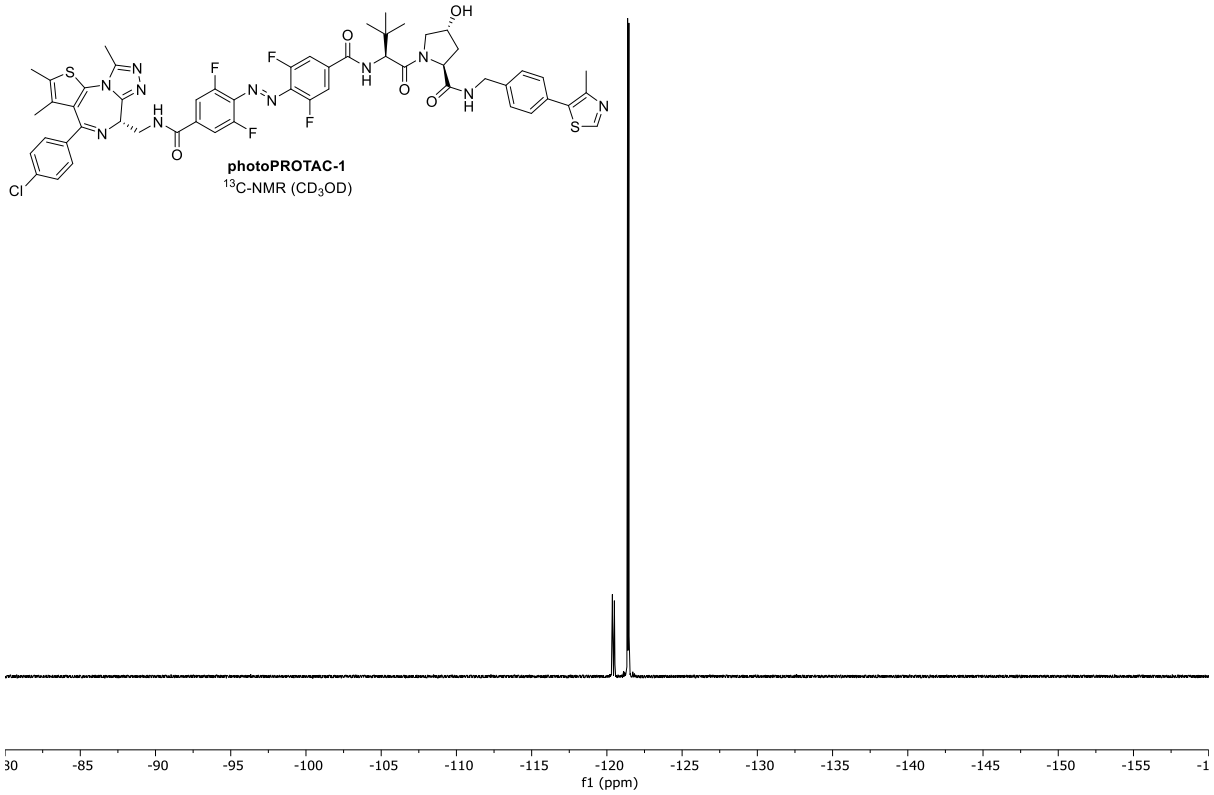


S4

^{19}F-NMR (CD_3OD)


photoPROTAC-1
1H-NMR (CD3OD)
f1 (ppm)


photoPROTAC-1
13C-NMR (CD3OD)
f1 (ppm)


photoPROTAC-1
13C-NMR (CD3OD)
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
-150
-155
f1 (ppm)

5. SI-references

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