Supporting Information

Photocontrolled Immunotherapy: A BODIPY-Caged MSA-2 for Spatiotemporal Activation of STING with Visible Light

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1. Experimental Section

Materials and Methods

Cell Culture and Plating

THP1-Dual™ monocytes (InvivoGen) were maintained in RPMI medium containing 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1x penicillin-streptomycin. For activation assays, 1 x 10^4 cells were seeded per well in clear, tissue-cultured 96-well plates and incubated at 37 °C, 5% CO_2, for 24 hours prior to treatment.

Preparation of Treatments

DMSO-stock BODIPY-MSA-2 and MSA-2, stored at -80 °C, were thawed and diluted to the appropriate starting concentration in THP1-Dual™ medium supplemented with 10 mM N-acetyl-L-cysteine and 10 mM sodium pyruvate immediately before use. All manipulations were performed under low-light conditions and outside of cell plates.

Photoactivation Protocol

Light-exposed treatments were irradiated outside the cell plate for 15 minutes using a 500-watt halogen work light (Hyper Tough™). A beaker containing room temperature water was placed between the light source and the treatment plate to prevent thermal buildup. Following irradiation, the treatments were immediately transferred to the cell plates and placed into the incubator under standard cell culture conditions. Dark-control aliquots were wrapped in aluminium foil after preparation and transferred directly to the cell plates without exposure to light.

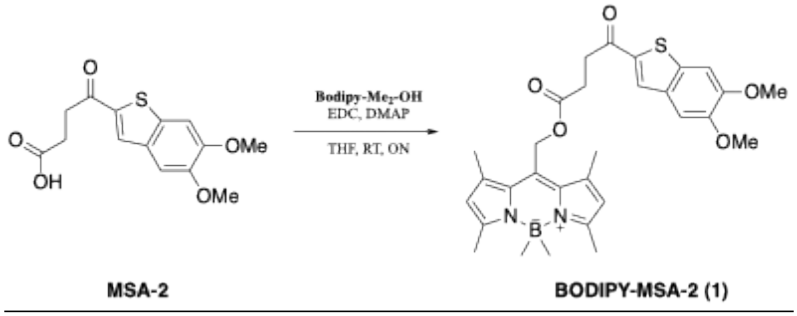
HPLC Assay

The HPLC method is at room temperature and utilizes a Hypersil™ BDS C18 column (130 Å pore size, 5 μm particle size, 150 × 4 mm inner diameter) and HPLC-grade water with 0.1% TFA (Solvent A) and HPLC-grade ACN with 0.1% TFA (Solvent B) as solvents. The method begins with a 5-minute flow of 95% Solvent A, followed by a -3% Solvent A per minute gradient for 30 minutes and a 9% Solvent A per minute gradient for 5 minutes, thereby returning to the starting solvent ratio.

Reporter and Viability Cells

After a 20-hour incubation period, IRF-dependent luminescence was quantified using the QUANTI-Luc™ assay (InvivoGen) according to the manufacturer's instructions. Cell plates were spun down for 5 minutes at 300 x g to separate the supernatant from the cells. Luminescence readings were recorded by an Agilent BioTek Synergy plate reader. Cell viability was measured in parallel using the CellTiter-Glo® (Promega) assay, with luminescence measured after a 30-minute incubation. The data from both assays were normalized to untreated controls and plotted and analysed on GraphPad Prism 10.

Synthesis of BODIPY-MSA-2



Bodipy-Me_2-OH[1,2] and MSA-2[3] were synthesized following literature procedures, and their structures were confirmed by ^{1}H NMR spectroscopy prior to use in further synthetic steps. A solution of MSA-2 (98 mg, 1 eq, 0.33 mmol) in THF (5 mL) was maintained under argon atmosphere. A solution of EDC (70 mg, 1.1 eq, 0.37 mmol) in THF (2 mL) was added dropwise followed by addition of 20 mol% DMAP (8.1 mg, 0.2 eq, 67 µmol). A solution of 4 (90 mg, 1 eq, 0.33 mmol) in THF (3 mL) was added to the reaction mixture. The reaction mixture was allowed to stir for 6 h. After consumption of the starting material, as judged by thin-layer chromatography (TLC) analysis. The solvent was evaporated in vacuo to obtain crude reaction mixture. The crude reaction mixture was purified by column chromatography on silica (0% to 40% EtOAc:hexanes) to obtain the product as an orange solid **1** (73 mg, 0.133 mmol, 40%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.25 (s, 1H), 7.23 (s, 1H), 6.05 (s, 2H), 5.38 (s, 2H), 3.98 (s, 3H), 3.95 (s, 3H), 3.34 (t, J = 6.5 Hz, 2H), 2.86 (t, J = 6.5 Hz, 2H), 2.44 (s, 6H), 2.37 (s, 6H), 0.19 (d, J = 3.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.6, 172.6, 153.3, 151.2, 137.3, 133.3, 131.3, 129.2, 122.8, 106.0, 103.7, 59.1, 56.3, 56.2, 33.6, 28.3, 16.7, 16.0. ^{11}B NMR (128 MHz, $CDCl_3$) δ -0.66. HRMS (ESI-MS): m/z calcd for $C_{30}H_{35}BN_2O_5S$ $[M+H]^+$: 547.2432, found 547.2439, error 1.28 ppm.

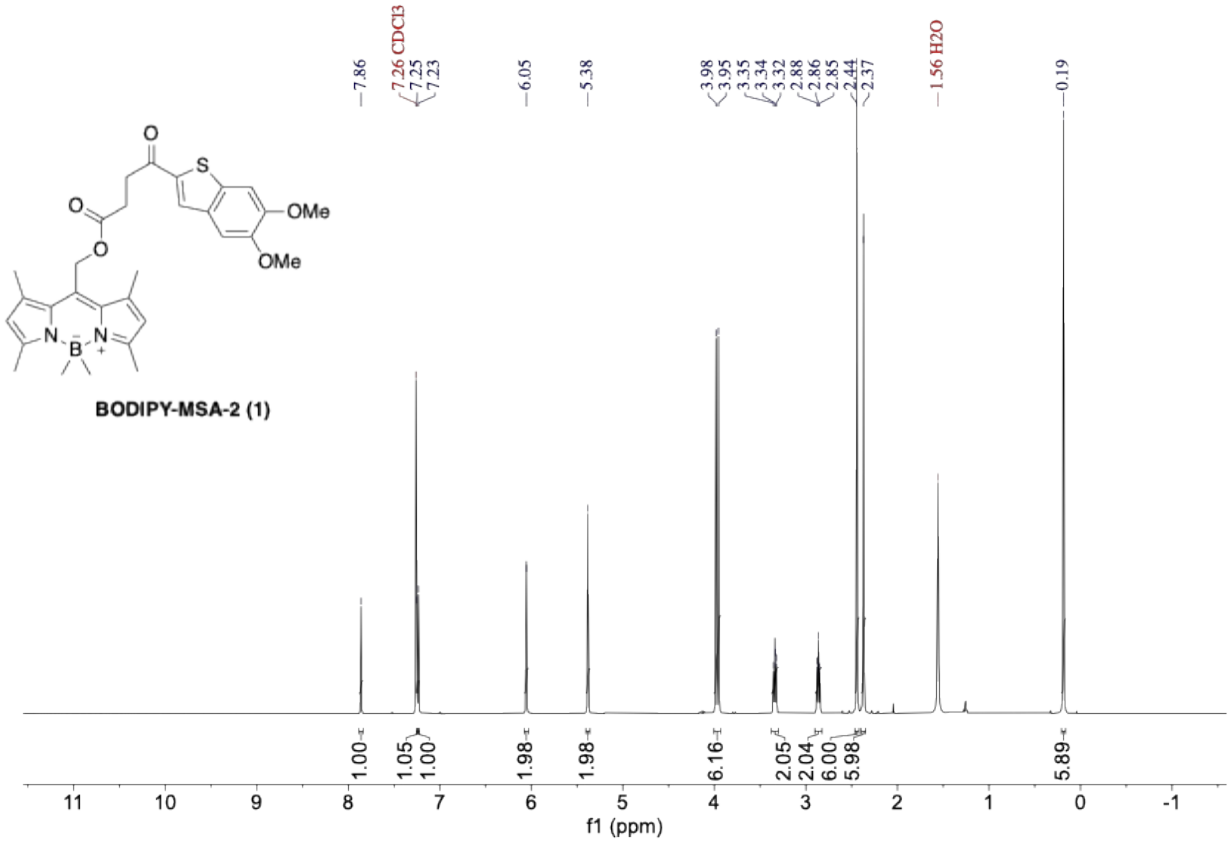


Figure S1: ^{1}H NMR ($CDCl_3$) for **BODIPY-MSA-2** (**1**)

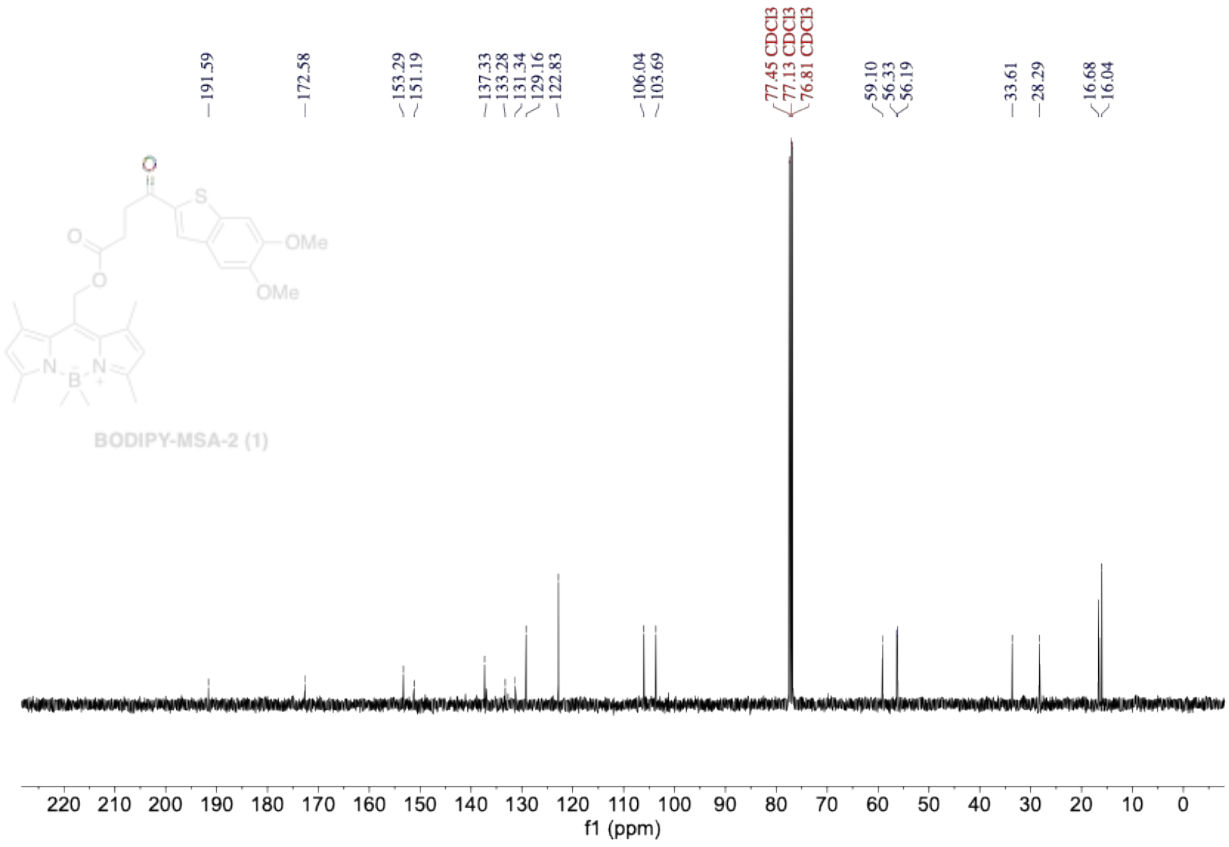


Figure S2: ^{13}C NMR ($CDCl_3$) for **BODIPY-MSA-2** (**1**)

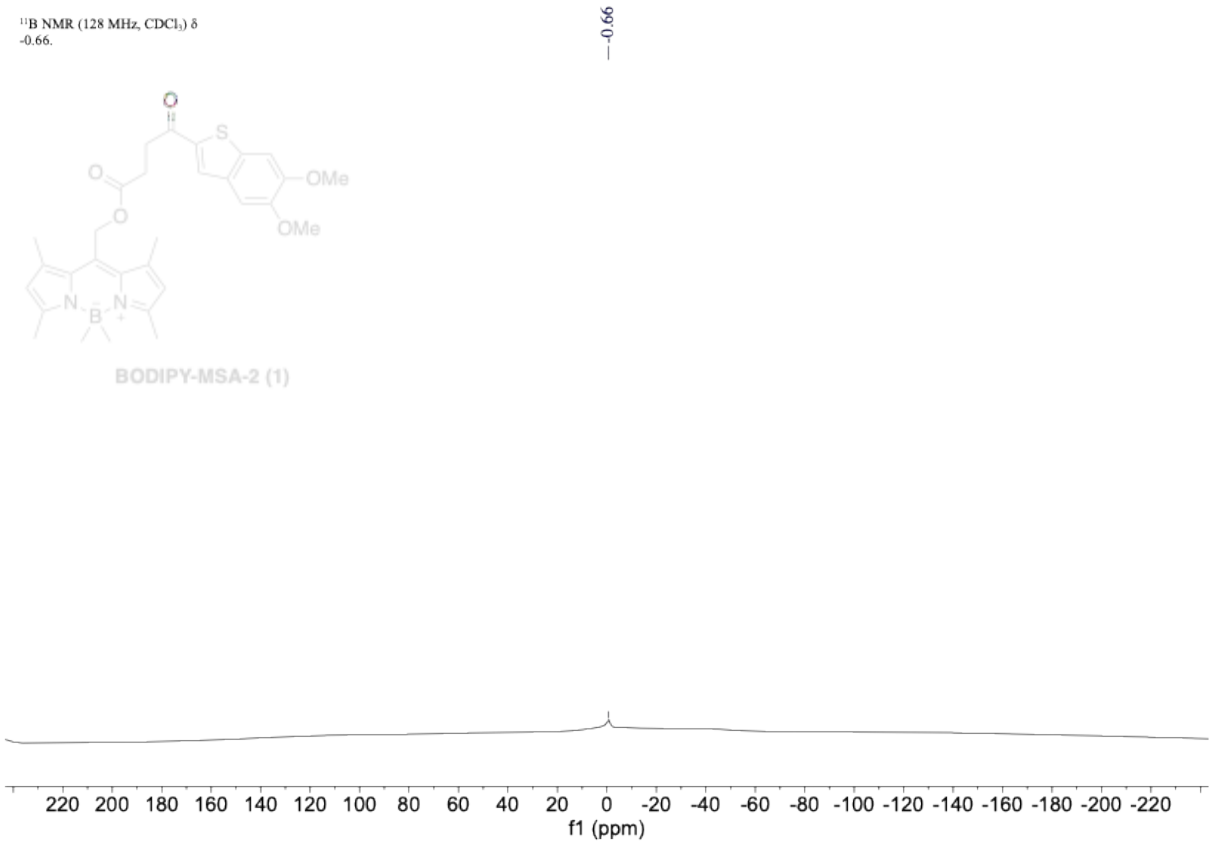


Figure S3: ^{11}B NMR ($CDCl_3$) for **BODIPY-MSA-2** (**1**)

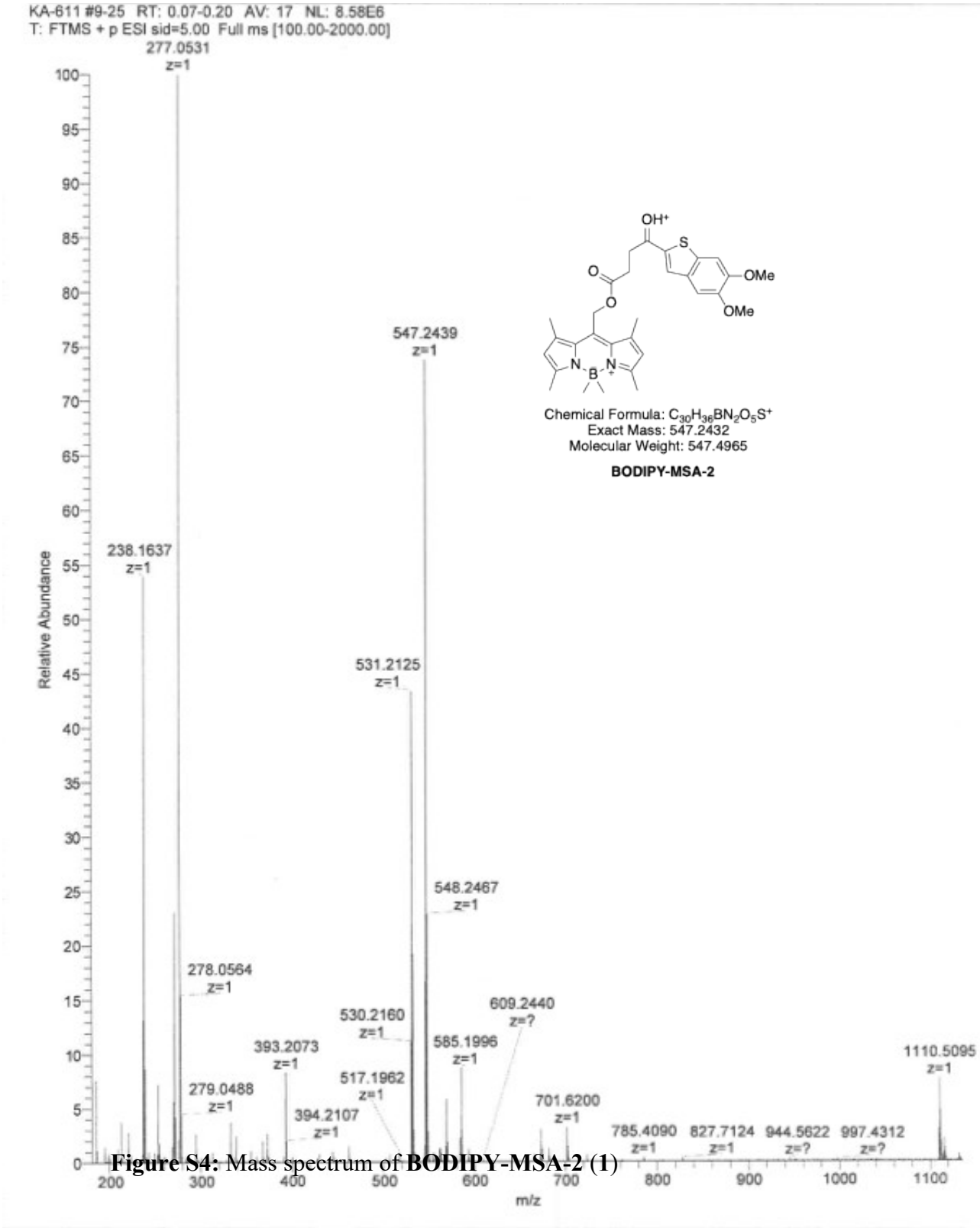
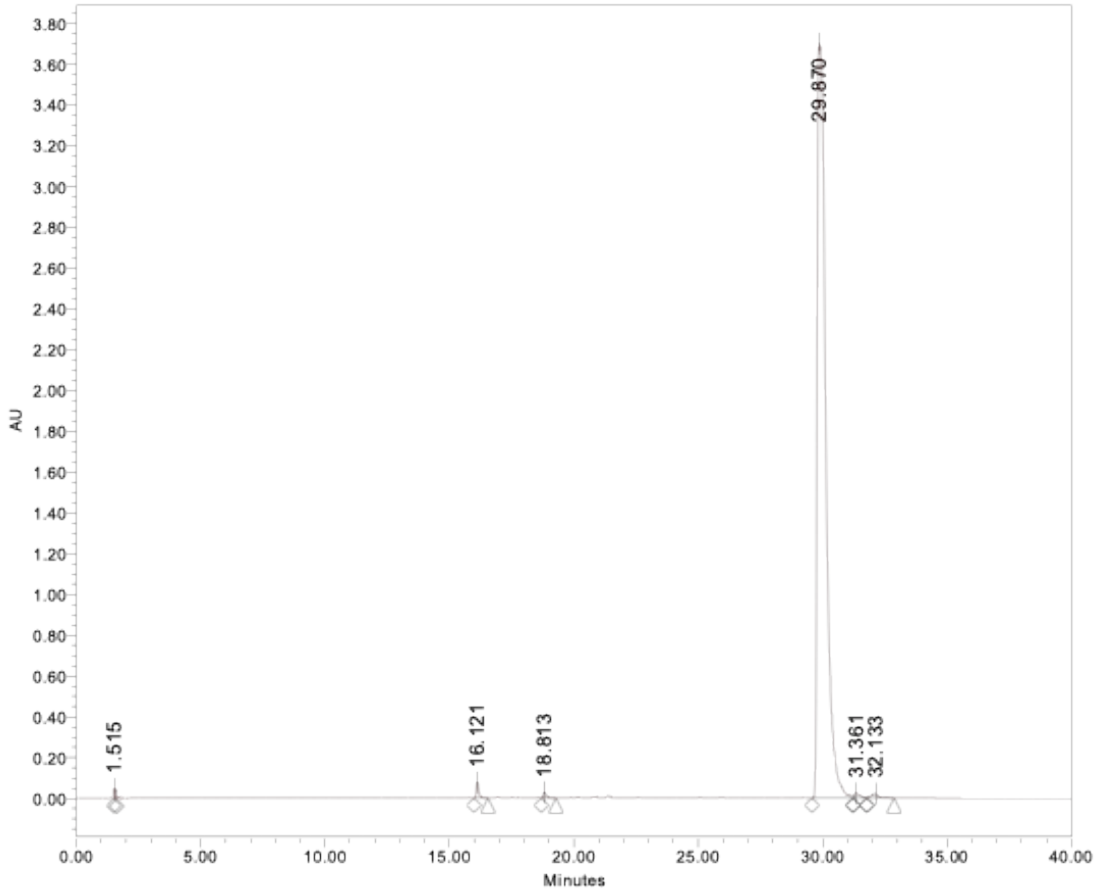


Figure S4: Mass spectrum of **BODIPY-MSA-2 (1)**

Empower 3

Peak Summary Report



Peak Summary with Statistics
Name:

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height
1	KA-611Dark	3	1	1.515	186179	0.20	48643
2	KA-611Dark	3	1	16.121	538828	0.59	76684
3	KA-611Dark	3	1	32.133	380712	0.41	18969
4	KA-611Dark	3	1	29.870	90210268	98.17	3700478

Reported by User: Jon Wilson (JonTWilson)
Report Method: Peak Summary Report
Report Method ID 59242
Page: 1 of 2

Project Name: Wilson lab
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Figure S5: HPLC Purity trace of **BODIPY-MSA-2 (1)**

Peak Summary with Statistics
Name:

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height
5	KA-611Dark	3	1	31.361	359949	0.39	22346
6	KA-611Dark	3	1	18.813	218291	0.24	26965
Mean				21.636			
Std. Dev.				11.965			
% RSD				55.30			

Figure S6: HPLC Purity trace of **BODIPY-MSA-2** (**1**)

References:

(1) Slanina, T.; Shrestha, P.; Palao, E.; Kand, D.; Peterson, J. A.; Dutton, A. S.; Rubinstein, N.; Weinstain, R.; Winter, A. H.; Klan, P. In search of the perfect photocage: structure-reactivity relationships in meso-methyl BODIPY photoremovable protecting groups. *J. Am. Chem. Soc.* **2017**, *139* (42), 15168-15175. DOI: 10.1021/jacs.7b08532.

(2) Toupin, N. P. a. A. K. a. S. P. a. P. J. A. a. F. L. J. a. R. E. a. P. I. a. W. A. H. a. K. BODIPY-Caged Photoactivated Inhibitors of Cathepsin B Flip the Light Switch on Cancer Cell Apoptosis. *ACS Chemical Biology* **2019**, *14* (12), 2833-2840.

(3) Pan, B.-S.; Perera, S. A.; Piesvaux, J. A.; Presland, J. P.; Schroeder, G. K.; Cumming, J. N.; Trotter, B. W.; Altman, M. D.; Buevich, A. V.; Cash, B.; et al. An orally available non-nucleotide STING agonist with antitumor activity. *Science (Washington, DC, U. S.)* **2020**, *369* (6506), eaba6098, 10.1126/science.aba6098. DOI: 10.1126/science.aba6098.