Native zinc catalyzes selective and traceless release of small molecules in β-cells

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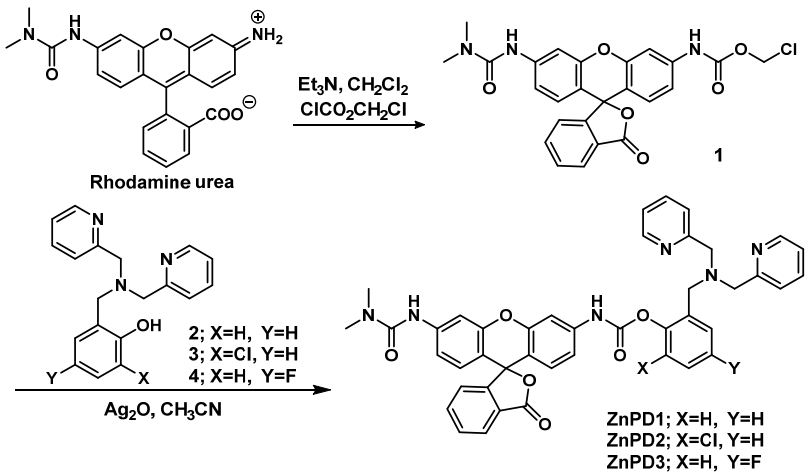
General procedures and materials.

All reagents were purchased and used as received from commercial sources without further purification. Reactions were performed in round-bottom flasks stirred with Teflon®-coated magnetic stir bars. Moisture and air-sensitive reactions were performed under a dry nitrogen/argon atmosphere. Moisture and air-sensitive liquids or solutions were transferred via nitrogen-flushed syringes. As necessary, organic solvents were degassed by bubbling nitrogen/argon through the liquid. The reaction progress was monitored by thin-layer chromatography (TLC) and ultra-performance liquid chromatography mass spectrometry (UPLC-MS). Flash column chromatography was performed using silica gel (60 Å mesh, 20–40 μm) on a Teledyne Isco CombiFlash Rf system. Analytical TLC was performed using Merck Silica gel 60 F254 pre-coated plates (0.25 mm); illumination at 254 nm allowed the visualization of UV-active material, and a phosphomolybdic acid (PMA) stain was used to visualize UV-inactive material. UPLC-MS was performed on a Waters ACQUITY UPLC I-Class PLUS System with an ACQUITY SQ Detector 2. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 Spectrometer (^{1}H NMR, 400 MHz; ^{13}C, 101 MHz) at the Broad Institute of MIT and Harvard. ^{1}H and ^{13}C chemical shifts are indicated in parts per million (ppm) relative to $SiMe_4$ (δ = 0.00 ppm) and internally referenced to residual solvent signals. NMR solvents were purchased from Cambridge Isotope Laboratories, Inc., and NMR data were obtained in $CDCl_3$ or DMSO. Data for ^{1}H NMR are reported as follows: chemical shift value in ppm, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, and m = multiplet), integration value, and coupling constant value in Hz. Tandem liquid chromatography-mass spectrometry (LCMS) was performed on a Waters 2795 separations module with a 3100 mass detector. High-resolution mass spectra were recorded on a JEOL AccuTOF LC-Plus 46 DART system at the department of chemistry instrumentation facility at the Massachusetts Institute of Technology and a Thermo Q Exactive Plus mass spectrometer system equipped with an HESI-II electrospray ionization source at Harvard Center for Mass Spectrometry at the Harvard FAS Division of Science Core Facility.

Synthesis of ZnPDs and ZnPD1ctrl

Synthesis of ZnPD1–3



Scheme S1. Synthetic route for **ZnPD1–3**.

Synthesis of Dimethylurea rhodamine-560 and Zinc-chelating ligand. Dimethylurea rhodamine-560 was prepared as described by Raines et al., and the spectroscopy data matched a literature report (*ACS Chem Biol.*, **2006**, *1*, 252–260). The zinc-chelating ligands (compound 1) were prepared as described by Nam et al., and the spectroscopy data matched reported values (*Chem. Commun.*, **2012**, *48*, 5449–5451). Compounds 2 and 3 were made the same way as compound 1.

Synthesis of Compound 1

Rhodamine urea (550 mg, 1.37 mmol) was mixed with anhydrous dichloromethane (15 mL, partially soluble) and cooled to −20 °C. Triethylamine (208 mg, 2.05 mmol) was added to this solution and stirred at this temperature for 5 min before adding chloromethyl chloroformate (212 mg, 1.64 mmol) dropwise. The color changed to dark red immediately. The reaction mixture was stirred at this temperature for 30 min, and the progress of the reaction was monitored by LC-MS. The compound was extracted with dichloromethane, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude residue, which was purified by flash chromatography on silica gel, eluting with 5% methanol in dichloromethane. The off-white solid product (compound 1, 42%) was obtained after purification and used for the next step to synthesize the ZnPDs. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 1H), 8.59 (s, 1H), 8.01 (d, J = 7.4 Hz, 1H), 7.79 (t, J = 7.2 Hz, 1H), 7.74 (d, J = 7.2 Hz, 1H), 7.69 (s, 1H), 7.57 (s, 1H),

7.28 (d, *J* = 7.4 Hz, 1H), 7.18 (t, *J* = 6.4 Hz, 2H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.64 (d, *J* = 8.6 Hz, 1H), 5.99 (s, 2H), 2.94 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.2, 155.8, 153.0, 151.6, 151.4, 151.2, 143.8, 140.8, 136.2, 130.7, 129.2, 128.2, 126.3, 125.2, 124.5, 116.1, 115.1, 114.0, 111.7, 106.4, 106.2, 82.6, 71.6, 36.7 (2C).

Synthesis of ZnPD1–3

Compound 1 (60 mg, 0.121 mmol) was placed in a 4 mL vial and dissolved in anhydrous acetonitrile (2 mL). Silver oxide powder (28.1 mg, 1.0 equiv.) was added followed by the corresponding Zn chelating group (compound 2–4, 1.1 equiv.) at room temperature. The resulting reaction mixture was stirred for 3 h at this temperature. The progress of the reaction was monitored by LCMS. The solvent was removed and the crude product was purified by flash chromatography on silica gel, eluting with 5% methanol in dichloromethane to produce a solid white compound.

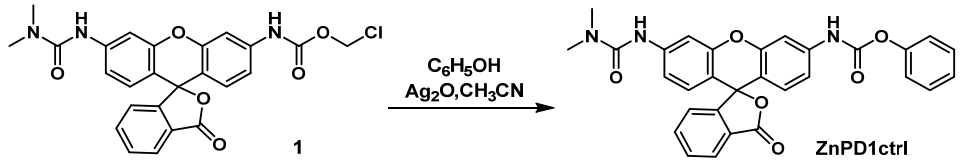
ZnPD1 (14% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (br, 1H), 8.52 (ddd, *J* = 5.0, 1.8, 0.9 Hz, 2H), 8.23 (br, 1H), 8.01 (dt, *J* = 7.4, 1.0 Hz, 1H), 7.67 (td, *J* = 7.4, 1.3 Hz, 1H), 7.64 – 7.53 (m, 4H), 7.45 (m, 3H), 7.37 – 7.27 (m, 2H), 7.22 – 7.10 (m, 6H), 7.00 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.77 – 6.61 (m, 3H), 3.97 (s, 4H), 3.82 (s, 2H), 3.03 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 164.5, 157.0, 155.6(2C), 153.4, 152.1, 152.0, 149.9, 147.5(2C), 141.7, 141.1, 138.4(2C), 135.3, 131.8, 129.9, 129.3, 129.0, 128.7, 128.5, 126.7, 125.9, 125.2, 124.7(2C), 124.1, 123.3, 123.2(2C), 115.6, 114.6, 113.6, 113.1, 107.5, 106.6, 83.2, 59.3(2C), 55.0, 36.7(2C). HRMS (Dart-TOF) (m/z): $[M+H]^+$ calculated for $C_{43}H_{37}N_6O_6$, 733.2775; found, 733.2769.

ZnPD2: The NMR spectrum was not obtained due to the instability of the compound. The compound decomposed in the NMR tube while attempting to obtain the spectrum.

ZnPD3 (11% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 8.51 (d, *J* = 4.9 Hz, 2H), 8.00 (d, *J* = 7.5 Hz, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.57 – 7.43 (m, 4H), 7.33 (d, *J* = 7.8 Hz, 2H), 7.19 – 6.92 (m, 7H), 6.76 (s, 1H), 6.66 (t, *J* = 9.1 Hz, 2H), 3.80 (s, 4H), 3.65 (s, 2H), 3.02 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 159.8 (*J* = 245 Hz), 158.1, 157.9, 155.5, 153.4, 152.4, 152.0, 151.9 (*J* = 9.5 Hz), 149.1, 148.7, 145.9 (2C), 145.8, 141.8, 141.2, 137.4, 137.0, 135.2, 129.9, 128.4 (*J* = 20.5 Hz) , 126.6, 125.1, 125.0, 124.8 (*J* = 8.4 Hz), 124.3, 124.1, 123.4, 122.8, 122.5, 118.0, 115.5, 115.2, 114.7, 113.6,

112.9, 107.4, 106.7, 83.2, 59.09, 55.29, 36.7 (2C). HRMS (Dart-TOF) (m/z): $[M+H]^+$ calculated for $C_{43}H_{36}N_6O_6F$, 751.2680; found, 751.2675.

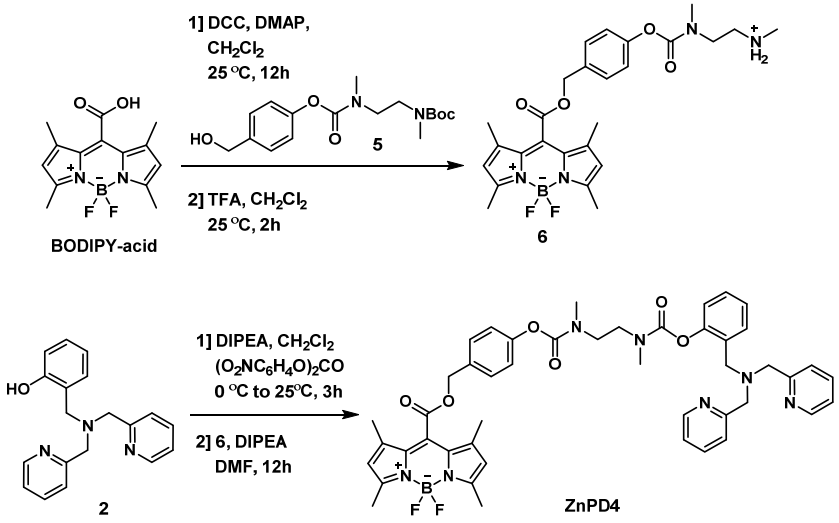
Synthesis of ZnPD1ctrl



Scheme S2. Synthetic route for ZnPD1ctrl**.**

ZnPD1ctrl (30% yield) was synthesized by following a method similar to that used for ZnPD1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.59 (s, 1H), 8.02 (dt, *J* = 7.5, 1.0 Hz, 1H), 7.80 (dt, *J* = 7.5, 1.2 Hz, 1H), 7.73 (dt, *J* = 7.5, 1.0 Hz, 1H), 7.68 (d, *J* = 2.1 Hz, 1H), 7.60 (d, *J* = 2.1 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.30 – 7.23 (m, 4H), 7.20 (ddd, *J* = 8.8, 7.4, 2.2 Hz, 2H), 6.75 (d, *J* = 8.7 Hz, 1H), 6.64 (d, *J* = 8.7 Hz, 1H), 2.93 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.7, 155.3, 152.7, 151.6, 151.1, 150.7, 150.3, 143.2, 140.9, 135.7, 130.2, 129.5(2C), 128.7, 127.7, 125.8, 125.6, 124.7, 124.0, 121.9(2C), 115.6, 114.6, 113.1, 111.2, 105.9, 105.6, 82.2, 54.9, 36.2. HRMS (Dart-TOF) (m/z): $[M+H]^+$ calculated for $C_{30}H_{24}N_3O_6$, 522.1665; found, 522.1660.

Synthesis of ZnPD4



Scheme S3. Synthetic route for ZnPD4.

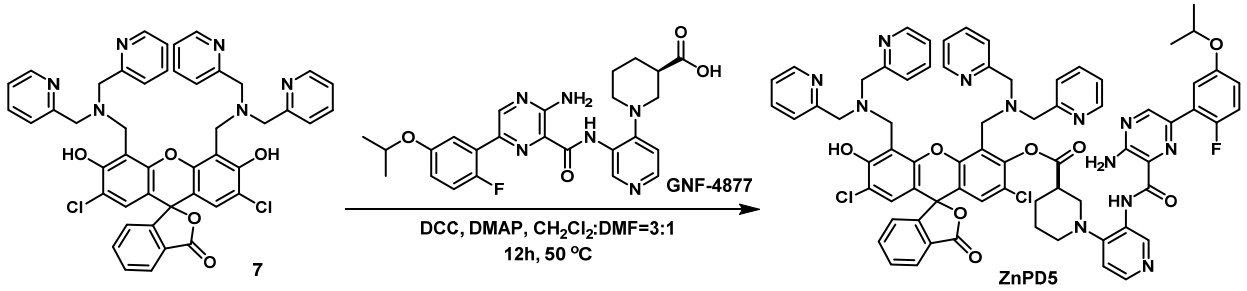
Synthesis of BODIPY-acid and linker 5. B-COOH-G was prepared as described by Changhua et al., and the spectroscopy data matched those previously reported (*J. Am. Chem. Soc*. **2017**, *139*, 10157−10163). The linker was prepared as described by Gillies et al., and the spectroscopy data matched literature values (*J. Am. Chem. Soc*. **2009**, *131*, 18327–18334).

Synthesis of ZnPD4.

Linker 5 (500 mg, 1.48 mmol) was dissolved in anhydrous dichloromethane (7.4 mL) followed by the addition of 4-dimethylaminopyridine (18 mg, 0.15 mmol), *N,N'*-dicyclohexylcarbodiimide (457 mg, 2.22 mmol), and BODIPY-acid (625 mg, 2.22 mmol). The reaction was stirred at room temperature for 12 h until the starting material was completely consumed. The reaction mixture was then diluted with dichloromethane, quenched with an aqueous solution of saturated $NaHCO_3$, and extracted with dichloromethane. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude residue, which was purified by flash column chromatography on silica gel, eluting with 5% methanol in dichloromethane. The orange foam product (compound 8) was obtained and carried forward to the next step. The ester (200 mg, 0.33 mmol) was dissolved in anhydrous dichloromethane (3.25 mL, 0.1 M) and then trifluoroacetic acid (370 mg, 3.25 mmol) was added to the reaction mixture. The solution was stirred for 2 h. The solvent was removed in vacuo, and dichloromethane was successively added and evaporated to remove residual trifluoroacetic acid and to provide the deprotected product. This product was carried over to the next step without further purification, wherein 4-nitrophenyl carbonate (119 mg, 0.39 mmol) and *N,N*-diisopropylethylamine (63 mg, 0.49 mmol) were added to the solution of zinc chelating ligand (99.3 mg, 0.33 mmol) in dichloromethane (3.25 mL) at 0 °C. The reaction mixture was slowly warmed up to room temperature over 3 h. *N,N*-diisopropylethylamine (168 mg, 1.30 mmol) and trifluoroacetic acid salt of compound 8 (0.33 mmol) in dimethylformamide were added to the reaction mixture and stirred for 12 h. The solvent was removed *in vacuo*, and the residue was purified by flash column chromatography eluting with 10% methanol in dichloromethane to give the pure fraction of ZnPD4 as a dark red solid (11% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 – 8.43 (m, 2H), 7.73 – 7.58 (m, 3H), 7.53 (d, *J* = 8.0 Hz, 2H), 7.48 – 7.34 (m, 2H), 7.21 –

7.09 (m, 6H), 6.99 (m, 1H), 6.02 (s, 2H), 5.36 – 5.33 (m, 2H), 3.85 – 3.74 (m, 4H), 3.74 – 3.53 (m, 6H), 3.21 – 3.10 (m, 3H), 3.06 (s, 3H), 2.51 (s, 6H), 2.00 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.1, 159.7, 157.8, 155.0, 154.9, 154.6, 152.1, 151.9, 150.1, 149.9 (2C), 141.3, 136.6 (2C), 131.5, 131.3, 130.8, 130.2, 128.1, 126.0, 125.9, 125.8, 122.9 (2C), 122.6, 122.5, 122.3 (2C), 122.1 (2C), 121.3 (2C), 68.1, 60.4, 53.6, 52.6, 52.4, 47.7, 47.0, 46.8, 35.6, 35.4, 14.9 (2C), 12.9 (2C). HRMS (Dart-TOF) (m/z): $[M+H]^+$ calculated for $C_{46}H_{49}BF_2N_7O_6$, 844.3805; found, 844.3840.

Synthesis of ZnPD5



Scheme S4. Synthetic route for ZnPD5.

Synthesis of Zinpyr-1 (7) and GNF-4877. Compound 7 was prepared as described by Burdette et al., and the spectroscopy matched literature reports (*J. Am. Chem. Soc*. **2001**, 123, 7831-7841). GNF-4877 was prepared as described by Shen et al., and the spectroscopy data agreed with previous reports (*Nat. Commun.* **2015**, 6, 8372).

Synthesis of ZnPD5

To a mixture of compound 7 (332.8 mg, 0.40 mmol) and GNF-4877 (100 mg, 0.20 mmol) in anhydrous dichloromethane (2.02 ml) and dimethylformamide (1.01 mL) was added *N*,*N*'-dicyclohexylcarbodiimide (62.5 mg, 0.3 mmol) and 4-dimethylaminopyridine (12.3 mg, 0.1 mmol). The reaction mixture was stirred for 12 hours at 50 °C. After evaporation of the solvent under reduced pressure the crude residue was chromatographed on silica gel with 10% methanol in dichloromethane to give the ester ZnPD5 (40 mg, 15.2 %) as a pale pink solid. ^{1}H NMR (400 MHz, DMSO-d_6, 330K) δ 10.31 (s, 1H), 9.32 (s, 1H), 8.63 (d, *J* = 2.1 Hz, 1H), 8.51 (d, *J* = 4.9, 2H), 8.32 (d, *J* = 5.4 Hz, 3H), 8.00 (d, *J* = 7.6, 1H), 7.83 (t, *J* = 7.5 Hz, 1H),

7.79 – 7.66 (m, 5H), 7.49 (t, *J* = 7.0 Hz, 2H), 7.42 (ddd, *J* = 6.5, 3.1, 1.3 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 7.29 – 7.24 (m, 3H), 7.24 – 7.14 (m, 4H), 7.10 – 7.03 (m, 2H), 6.99 (dt, *J* = 9.1, 3.5 Hz, 1H), 6.69 (d, *J* = 2.2 Hz, 1H), 6.56 (s, 1H), 4.58 (septet, *J* = 6.0, 0.9 Hz, 1H), 4.15 (s, 2H), 4.08 – 3.83 (m, 6H), 3.75 (s, 4H), 3.49 (d, *J* = 10.4 Hz, 1H), 3.25 (buried in the signal of H_2O, 1H) 2.97 (t, *J* = 12.8 Hz, 1H), 2.92 – 2.75 (m, 2H), 2.01 (br, 1H), 1.82 (br, 2H), 1.57 (br, 1H), 1.23 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6, 298K) δ 169.8, 168.0, 163.5, 157.9 (2C), 157.2 (2C), 155.7, 154.3, 153.9, 153.6 (*J* = 239 Hz), 151.0, 149.5, 148.8 (2C), 148.5, 147.9 (*J* = 9.6 Hz), 147.8, 147.7, 146.9, 146.2, 142.2, 137.1 (2C), 135.9 (2C), 135.8, 135.0 (*J* = 3.4 Hz), 130.7, 128.2, 126.9, 126.4, 125.7, 125.3, 124.6 (*J* = 14 Hz), 123.9, 123.5, 123.1 (2C), 122.8 (2C), 122.6 (2C), 122.1, 122.0 (2C), 121.3, 117.8, 117.2, 116.8 (*J* = 24.0 Hz), 116.6, 115.9 (*J* = 6 Hz)), 115.1, 112.0, 109.3, 81.2, 69.8, 59.5, 59.0, 58.6, 52.5, 50.2, 48.9, 48.6, 41.6, 26.5, 24.4, 21.7, 21.6. HRMS (Dart-TOF) (m/z): $[M+H]^+$ calculated for $C_{71}H_{62}C_{l2}FN_{12}O_8$, 1299.4175; found, 1299.4169.

Cell culture

All cells were cultured in a humidified 5% CO_2 atmosphere at 37 °C. HEK293T (ATCC) and PANC-1 (ATCC) were cultured in Dulbecco's Modified Eagle's Medium (Life Technologies) supplemented with 10% fetal bovine serum (FBS; Life Technologies) and 1x penicillin/streptomycin (Life Technologies). INS-1E cells were cultured in RPMI 1640 medium (Life Technologies) supplemented with 10% FBS, 1× penicillin/streptomycin, 1 mM sodium pyruvate (Life Technologies), and 3.5 µL/L BME (Sigma). Alpha TC1 clone 6 cells were cultured in Dulbecco's Modified Eagle's Medium, low glucose (Gibco Cat. No. 11885-084) medium supplemented with 10% HyClone FBS and 1× penicillin/streptomycin. Human islet cells were maintained in CMRL 1066 (Life Technologies) containing 10% FBS, 1× penicillin/streptomycin, and 2 mM L-glutamine. Cells were continuously maintained at <90% confluency. All cell lines were sourced commercially or were functionally validated. Cells were periodically tested for mycoplasma contamination using the MycoAlert PLUS Mycoplasma Detection Kit (Lonza).

Beta-cell specific fluorophore release

In a 96-well plate, INS-1E (30,000 cells), HEK293T (12,000 cells), alpha TC1 clone 6 (30,000 cells), and PANC-1 (20,000 cells) were plated per well and cultured for 24 h at 37 °C with 5% CO_2. Compounds (DA-ZP1, DA-FC, ZnPD1, ZnPD4, ZnPD5, and ZnPD1ctrl) at the indicated concentration in the corresponding cell culture medium containing 2× DAPI were incubated for 1 h inside the incubator. The medium containing compound was removed and washed with 3× 100 µL of fresh medium, and finally, another 100 µL of fresh, dye-free medium was added. The cells were immediately imaged using a high-content fluorescence microscope (Molecular Devices or Operetta Phenix) in both the DAPI and FITC channels at 20X magnification. Images were analyzed using the MetaXpress or Harmony software and data were plotted using GraphPad Prism 6.

Beta-cell specific staining of human islets

Approximately 30,000 freshly dissociated human islet cells per well were plated in a 96-well format and incubated for 24 h at 37 °C with 5% CO_2. Cells were incubated with DA-ZP1 (5 µM) at the indicated

concentration in the human islet cell culture medium for 1 h in the incubator. Cells were then washed with 2× 100 µL of fresh medium before fixing and permeabilizing with cold methanol for 1–2 min following a literature protocol (Jamur *et al.*, 2010). Cells were then immunostained with rat anti-C-Peptide antibody (Cell Signaling) overnight at 4 °C and subsequently stained with Alexa Fluor 647 anti-rat (ThermoFisher) and 1× DAPI (ThermoFisher) for 1 h. The plates were imaged using the Phenix high-content confocal microscope (Perkin Elmer) with DAPI as the nuclear counterstain.

DA-ZP1- or ZnPD4-assisted β-cell sorting

Upon receipt, islets were centrifuged at 1000 rpm for 1 min and resuspended in supplemented CMRL 1066 medium. Islets were then transferred to 10 cm culture dishes and cultured overnight to 24 h. Healthy islets were handpicked and washed with DPBS. For DA-ZP1 or ZnPD4 staining, islets were dissociated into single cells by TrypLE. Briefly, 1 mL TrypLE was added to human islet pellet and incubated at 37 °C for 12 min by mixing the tube every 3-4 min. At the end of incubation, TrypLE was neutralized by adding 9 mL DMEM HG containing 10% FBS. The cell suspension was filtered using a 30 μm filter to remove any aggregates, counted on a hemocytometer, and pelleted by spinning cells at 1200 rpm for 5 min. Cells were washed with CMRL 1066 final wash/culture medium and resuspended in the final wash/culture medium with DA-ZP1 or ZnPD4 (160 nM) for 30 min at 37 °C. At the end of the incubation period, cells were washed and the cell pellet was resuspended in the final wash/culture medium. DA-ZP1 or ZnPD4 positive and negative cells were sorted by FACSAria cell sorter (BD Biosciences, Joslin Flow Cytometry Core). Sorted cells were spun at 1200 rpm for 5 min and washed with DPBS, then fixed in 4% PFA for 15 min at room temperature. Cells were washed and embedded in agarose and paraffin, sectioned and used for immunostaining as previously described by El Ouaamari et al., (*Cell Metab*. **2016**, 23, 194-205). Analysis of flow cytometry data was completed using FlowJo 10.4.2 (FlowJo LLC, Ashland, OR). The gating strategy is shown in Figures S3 and S7. The donor demographic information is summarized in Table S1.

Kinetics of DA-ZP1 and ZnPD4 in cells

The cells were plated in black-walled, clear-bottom 96-well plates coated with poly-D-lysine (PDL, Sigma Aldrich) for 1 h and washed with 200 µL of PBS/well. INS-1E and αTC-1.6 cells were plated at 30,000

cells/well, and PANC-1 and HEK293T cells were plated at 11,250 cells/well. After plating, the cells were incubated overnight at 37 °C. DAZP1 or ZnPD4 solutions were prepared in microcentrifuge tubes using the respective, dye-free cell mediums: RPMI 1640, DMEM + GlutaMAX, or DMEM (low glucose). The medium in the 96-well plates was removed by *gently* inverting the plates upside down. Any medium remaining in the wells was not removed. DAZP1 or ZnPD4 solutions (10 μM–10 nM) in dye-free medium were added to the wells (100 μL/well). After the solutions were added, the plates were incubated for 1–25 h at 37 °C, after which time the plates were gently inverted to remove the medium. Any residual medium was removed with a multichannel pipette. The wells were then gently washed twice with reconstituted dye-free cell medium (100 μL/well). DAPI was then added to the wells in dye-free cell medium (100 μL/well), and the plates were incubated at 37 °C for 30 min. DAPI was removed by gently inverting the plates, and the residual medium was removed with a multichannel pipette. The wells were washed with 50 μL/well of dye-free INS-1E/PANC-1 medium. Finally, 100 μL/well of dye-free cell medium was added to wells, and the plates were sealed for live-cell imaging.

Cargo release detection by LC-MS

Samples of 1 × 10^6 cells were treated with compound (5 μM) in a 6-well plate for the indicated duration before collecting by treatment with trypsin. Cells were re-suspended in 1 mL PBS and pelleted down at 4000×g for 2 min. The supernatant was removed carefully keeping the residual PBS along with the cell pellet. Cells were briefly vortexed and 10 μL DMSO was added before vortexing vigorously for 15 s. The cell suspension was flash-frozen in liquid nitrogen followed by thawing and vortexing for 15 s. This process was repeated twice. A 50-μL aliquot of acetonitrile containing 2 μM spautin as an internal standard was added and the mixture was vortexed for 30 s. The suspension was centrifuged at 10000×g for 1 min and then the supernatant was collected for mass spectrometric analysis.

Fluorescence spectroscopic studies on ZnPDs

The compound in PBS was taken in a 96-well microplate (250 µL, 10 µM per well, Nunc 96 Well Plates) and incubated with different concentrations of $ZnSO_4$ (0-1 mM). Reaction kinetics were monitored by measuring the fluorescence of the activated fluorescein dye (λ_{ex} = 490 nm, λ_{em} = 522 nm) over 20 h.

Stability of ZnPD5 in cell culture media

Samples of the compound in INS-1E and human islet media (250 µL, 10 µM per well) alone or in the presence of varying concentrations of $ZnSO_4$ (125-1000 µM) were incubated and the stability was monitored by measuring the fluorescence signal (λ_{ex} = 490 nm, λ_{em} = 522 nm) for over 12 h.

Cellular localization

Localization of DA-ZP1 release was performed following a reported cell-painting method (*Nat. Protoc.* **2016**, 11, 1757). In a typical experimental procedure, INS-1E cells were treated with 5 µM DA-ZP1 for 1 h in a 96-well plate. The media was removed and 60 µL/well MitoTracker (Invitrogen, cat. no. M22426) solution containing 2× DAPI was added and incubated for 30 min in the dark. To the same well, 20 µL 16% PFA was added and incubated for an additional 20 min in dark. Cells were then washed twice with 100 µL HBSS and then processed for imaging. The cells were imaged by confocal microscopy using DAPI, FITC, and deep red (647 nm) channels.

Effect of the metal chelator

INS-1E cells were first incubated in media containing either DMSO or 25 µM TPEN for 30 min followed incubating with DA-ZP1 (5 µM) or treated with 4% PFA for fixation. DA-ZP1 treated live cells were processed for imaging after 30 min of incubation. The fixed cells were incubated with ZnPD5 (5 µM) in PBS containing 1× DAPI for 3 h. Cells were then imaged under a fluorescence microscope using DAPI and FITC channels.

Intact islet staining

Intact islet cells were pipetted up and down 5-7 times in culture media using 1 mL tips and then portioned (60-80 islets per condition) into 1 mL microtubes. Islets were incubated with ZnPD5 at 5 μM for 3 h inside the incubator with occasional mixing. Cells were then spun down (200 RCF, 2 min) and the supernatant was carefully removed. 300 μL of TrypLE was added to each tube and incubated at 37 °C for 12 min with occasional mixing. Culture media (700 μL) was added to each tube and pipetted up and down 7-10 times using a 1 mL pipet. Cells were then spun down at 200 RCF for 5 min and the supernatant was carefully removed. Cells were then resuspended in 300 μL media and transferred to a pre-coated 96-well plate in two replicates. Cells were allowed to adhere by culturing for 12 h and then fixed with 4% PFA for 20 min and subsequently permeabilized with 0.2% Triton-X for 20 min. Cells were then processed for primary and secondary antibody staining following the protocol as discussed earlier. Cells were imaged by using fluorescence microscopy under DAPI, FITC, and 647 nm channels.

Immunostaining

Sections of agar and paraffin-embedded cells were stained as previously described (*J. Diabetes Res.* **2015***, 2015,* 450128). Primary antibodies to all antigens and appropriate secondary antibodies with their working dilutions are listed in Table S2. Digital images were taken with an AXIO Imager A2 upright microscope equipped with an X-Cite series 120Q light source, Axiocam 512 color camera, and Zen 2.3 lite software. Images were overlaid and hormone-positive cells counted using ImageJ software. ≥ 5,000 cells were counted per group (n=3 replicate slides).

Human Islet proliferation

Human islets were dissociated into a single cell suspension using TrypLE and plated at 10,000 islet cells per well in coated 384-well plates. Islet cells were cultured in FBS-supplemented CMRL 1066 medium with 10 μM 5-ethynyl-2'-deoxyuridine (EdU) and incubated at 37 °C with 5% CO_2. Every 2 days, cells were treated with DMSO and ZnPD5 in duplicate at doses ranging from 312.5 nM to 2.5 μM. After 6 days, cells were fixed using 3.7% PFA, permeabilized using 0.2% Triton X-100, and copper-catalyzed click chemistry

was performed to visualize proliferating cells using Alexa Fluor 488 azide (ThermoFisher). Cells were subsequently immunostained with rat anti-C-peptide antibody and goat anti-rat Alexa Fluor 647 (ThermoFisher) to mark β-cells along with DAPI as a nuclear counterstain. For each well, 16 fields were acquired using the Opera Phenix™ (PerkinElmer) and image analysis was performed using Harmony® Software (PerkinElmer) to calculate β-cell proliferation.

Table S1. Donor Information

	Batch-1	Batch-2	Batch-3	Batch-4	Batch-5
Donor ID	SAMN09767131	SAMN09929411	SAMN10391370	SAMN10602026	SAMN12496804
Purity	80%	90%	90%	90%	90%
Viability	96%	95%	90%	90%	92%
Health	Nondiabetic	Nondiabetic	Nondiabetic	Nondiabetic	Nondiabetic
Age	29	37	48	51	40
Gender	M	M	M	F	F
BMI	24.1	29	24.4	28.5	30.4
Ethnicity/Race	Hispanic/Latino	Black or African American	Hispanic	White	White
Cause of Death	Cerebrovascular/ stroke	Head Trauma	Head Trauma	Cerebrovascular/ stroke	Cerebrovascular/ stroke
Source	Southern California Islet Cell Resources Center	The Scharp-Lacy Research Institute	University of Miami	University of Miami	University of Miami

Table S2. Primary antibodies to all antigens and appropriate secondary antibodies

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rat anti-C-Peptide (1:100)	Developmental Studies Hybridoma Bank	GN-ID4; RRID: AB_2255626
Guinea Pig anti-Insulin (1:400)	Abcam	ab7842; RRID: AB_306130
Mouse anti-Glucagon (1:10,000)	Sigma	G2654; RRID: AB_259852
Rat anti-Somatostatin (1:100)	Abcam	ab30788; RRID: AB_778010
Goat anti-PPY (1:150)	Novus	NB 100-1793, RRID: AB_526299
Donkey anti-Guinea Pig AF594 (1:400)	Jackson ImmunoResearch	706-586-148; RRID: AB_42340475
Donkey anti-Guinea Pig AF488 (1:400)	Jackson ImmunoResearch	706-546-148; RRID: AB_2340473
Donkey anti-Rat AF488 (1:400)	Jackson ImmunoResearch	712-546-153; RRID: AB_2340686
Donkey anti-Mouse AF594 (1:400)	Jackson ImmunoResearch	715-586-150; RRID: AB_2340857
Donkey anti-Mouse AF488 (1:400)	Jackson ImmunoResearch	715-546-150; RRID: AB_2340849
Donkey anti-Goat AF594 (1:400)	Jackson ImmunoResearch	705-586-147; RRID: AB_2340434
DAPI, dilactate	Sigma	D9564
CMRL 1066 final wash/culture medium	Mediatech	99-785-CV
Supplemented CMRL 1066	Mediatech	99-603-CV
TrypLE	Thermo Fisher Scientific	12604-013
DMEM HG (no phenol red)	Thermo Fisher Scientific	21063029
Penicillin-streptomycin	Corning	30-002-CI
FBS	Thermo Fisher Scientific	10437028

Dose and Time-dependent of beta-cell specific hydrolysis with DA-ZP1

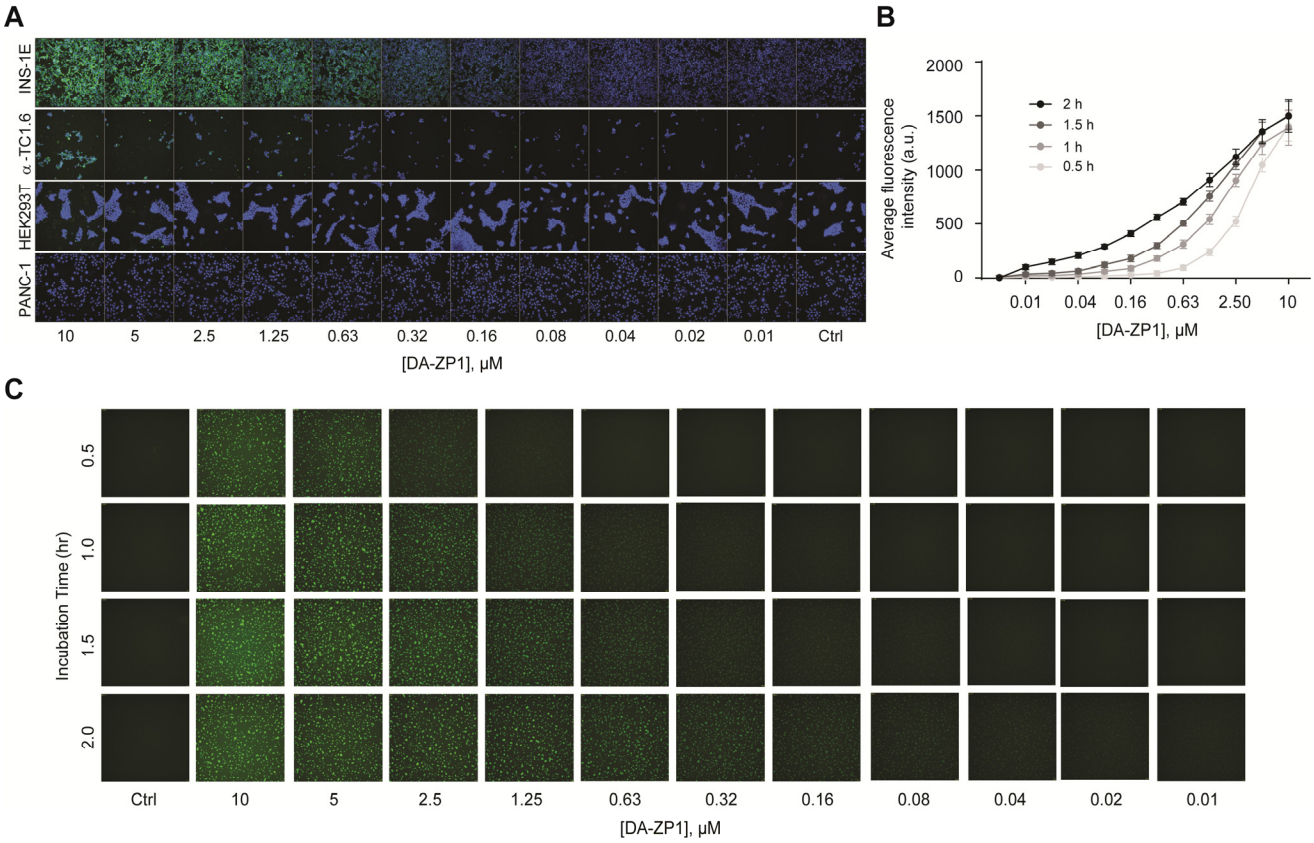


Figure S1. (A) β-cell-specific hydrolysis of DA-ZP1 unmasks fluorescence in a dose-dependent manner after 1 h of compound incubation at different concentrations. Each panel represents a merged FITC and DAPI channel. **(B)** Fluorescence release kinetics in INS-1E cells at increasing concentrations of DA-ZP1. **(C)** Time-dependent unmasking of DA-ZP1 in INS-1E cells imaged with the FITC channel.

Evaluation of the intracellular localization of DA-ZP1 and Zn(II) depletion

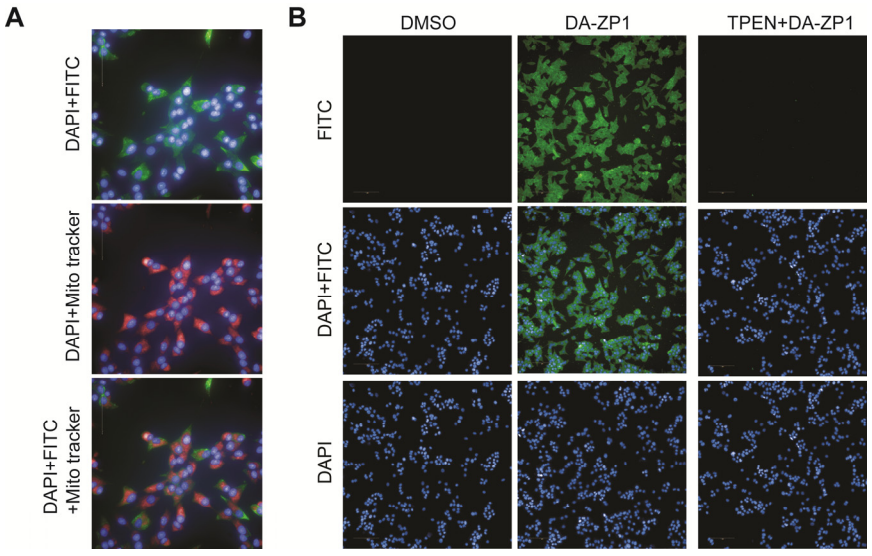


Figure S2. (A) Cellular localization of ZP1 in INS1E cells. Cells were treated with DA-ZP1 (5 μM) for 1 h followed by fluorescence imaging using DAPI and MitoTracker as counterstaining for nuclei and mitochondria, respectively. **(B)** Fluorescence image showing inhibition of DA-ZP1 (5 μM) staining after 30 min of incubation with INS1E cells in the presence of metal chelator (TPEN, 25 μM).

Sorting human pancreatic beta cells using DA-ZP1

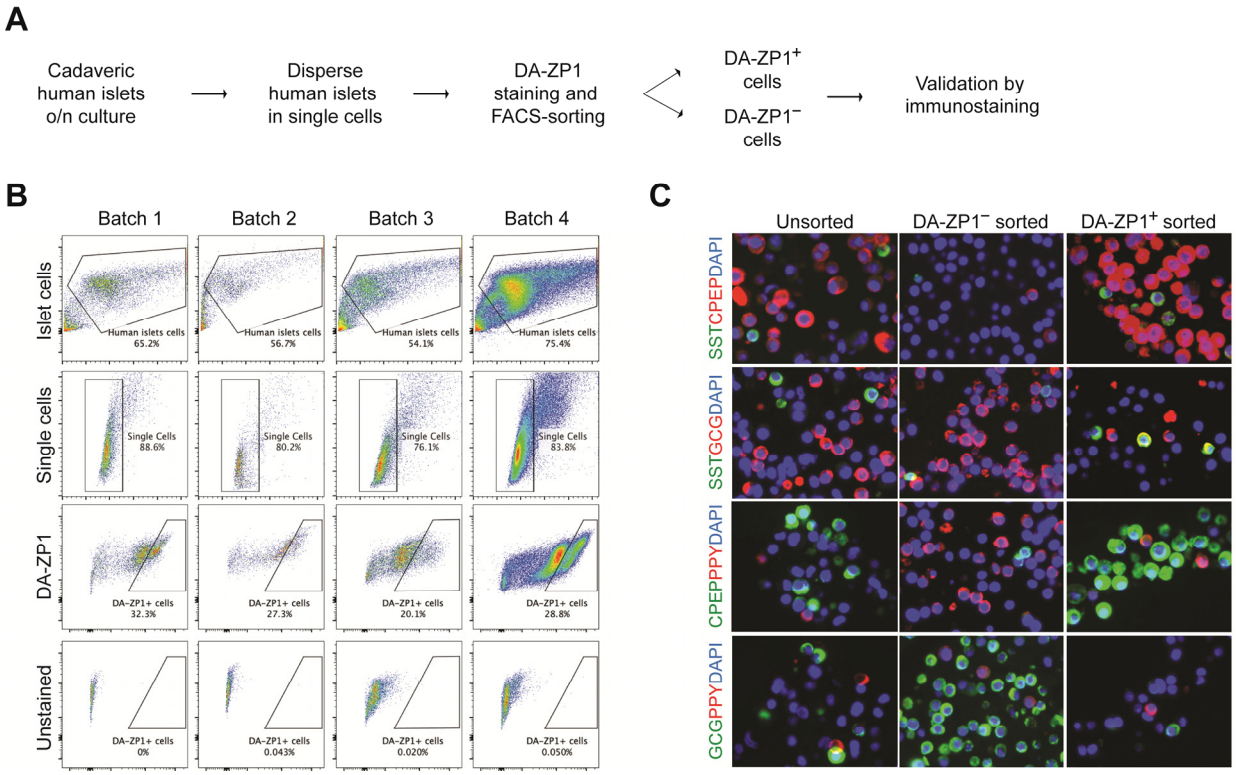


Figure S3. **(A)** Overview of DA-ZP1-based experimental approach for human pancreatic islets cell sorting. **(B)** Representative flow plots show the sorting of human islets cells from four different donors. The gating strategy is shown in each plot by labeling the gated population. Cell debris was excluded by forwarding versus side scatter (FSC-A vs SSC-A) and singlets were identified by forward scatter area versus wide (FSC-A vs FSC-W). DA-ZP1 stained cells were isolated according to unstained samples. **(C)** DA-ZP1^{+} and DA-ZP1^{-} human islet cells were collected by FACS. Cells were embedded in agarose and paraffin for sectioning. Slides were stained for islet hormones. Representative images show insulin (CPEP), glucagon (GCG), somatostatin (SST), pancreatic polypeptide (PPY) staining in unsorted, DA-ZP1^{+}, DA-ZP1^{-} cell populations. Nuclei stained with DAPI (blue).

Zn(II)-mediated hydrolysis of ZnPD1-3 and ZnPD1ctrl

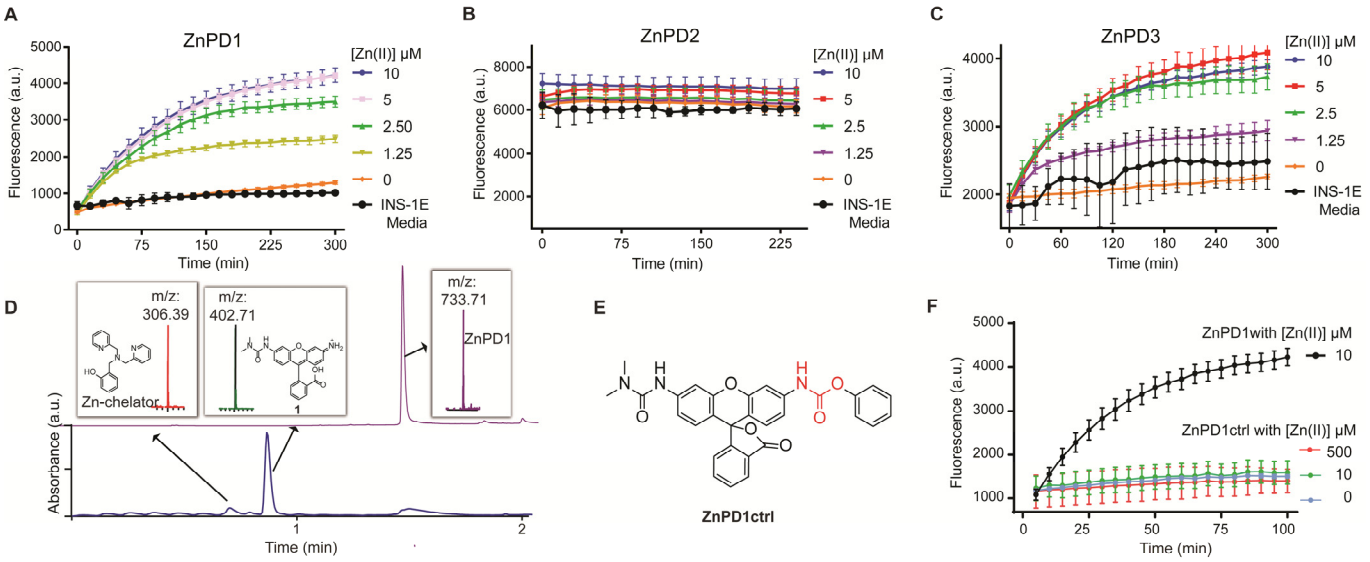


Figure S4. **(A)** Zn(II)-mediated hydrolysis of ZnPD1 (10 μM) in PBS buffer (pH 7.4). **(B)** Zn(II)-mediated hydrolysis of ZnPD2 (10 μM) in PBS buffer (pH 7.4). **(C)** Zn(II)-mediated hydrolysis of ZnPD3 (10 μM) in PBS buffer (pH 7.4). **(D)** LC-MS chromatogram of Zn(II)-mediated (10 μM) hydrolysis of ZnPD1 (10 μM) in PBS buffer (pH 7.4). **(E)** Chemical structure of ZnPD1ctrl. **(F)** A comparison of Zn(II)-mediated hydrolysis between ZnPD1 (10 μM) and ZnPD1ctrl (10 μM) in PBS buffer (pH 7.4). Excitation and emission wavelength of 490 nm and 522 nm were used for the studies.

Dose-dependent of beta-cell specific hydrolysis of ZnPD1

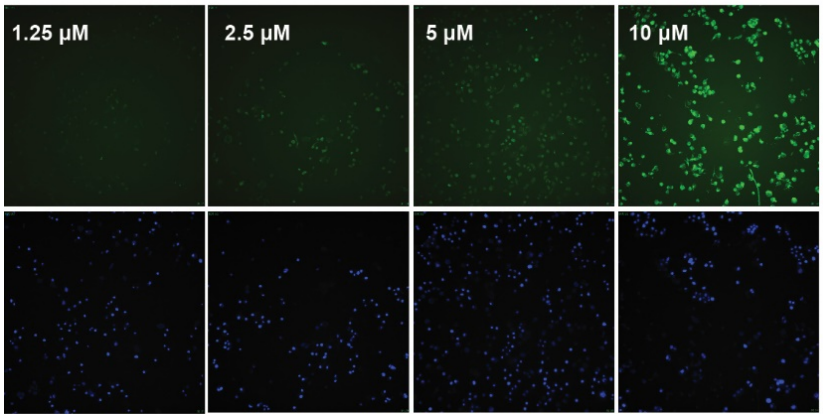


Figure S5. Dose-dependent release of rhodamine urea from ZnPD1 in INS-1E cells after 1 h of incubation. Top and bottom panels represent images acquired in FITC and DAPI channels, respectively.

Dose and Time-dependent of beta-cell specific hydrolysis with ZnPD4

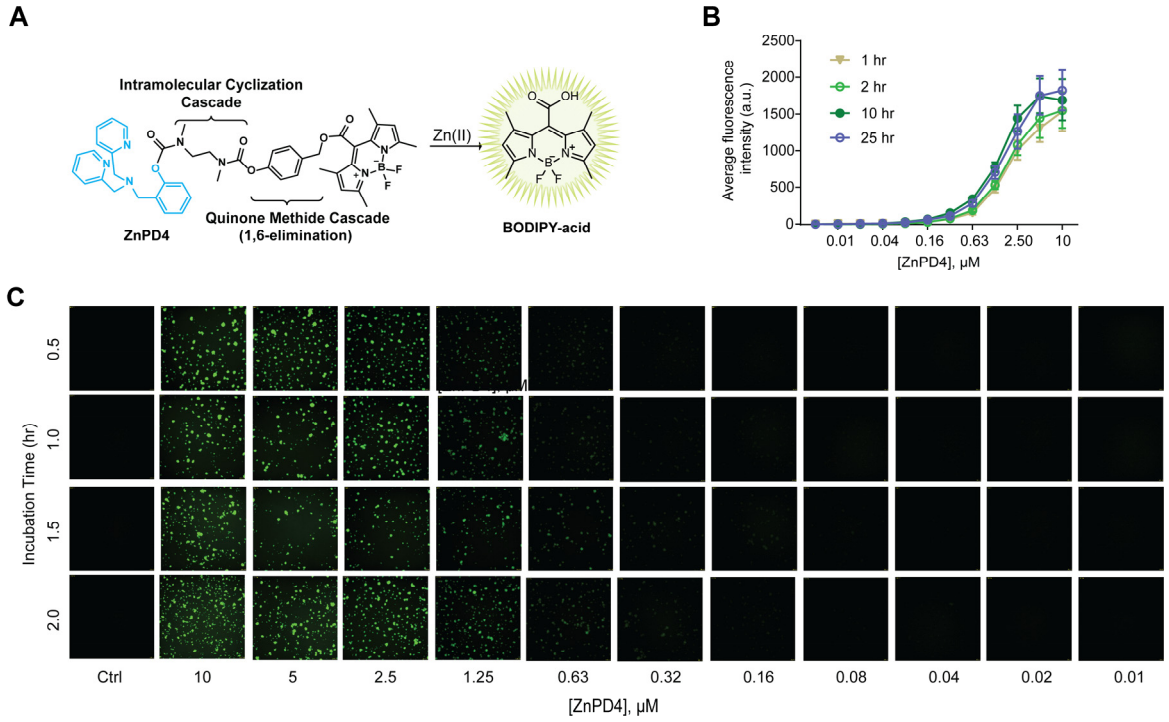


Figure S6. (A) Proposed mechanism of Zn(II)-mediated hydrolysis of ZnPD4. **(B)** Time-dependent release of BODIPY acid (compound 2) from ZnPD4 in INS-1E cells by Zn(II)-mediated hydrolysis followed by self-immolation. **(C)** Representative images of the time-dependent release of BODIPY acid from ZnPD4 in INS-1E cells imaged with the FITC channel.

Sorting human pancreatic beta cells using ZnPD4

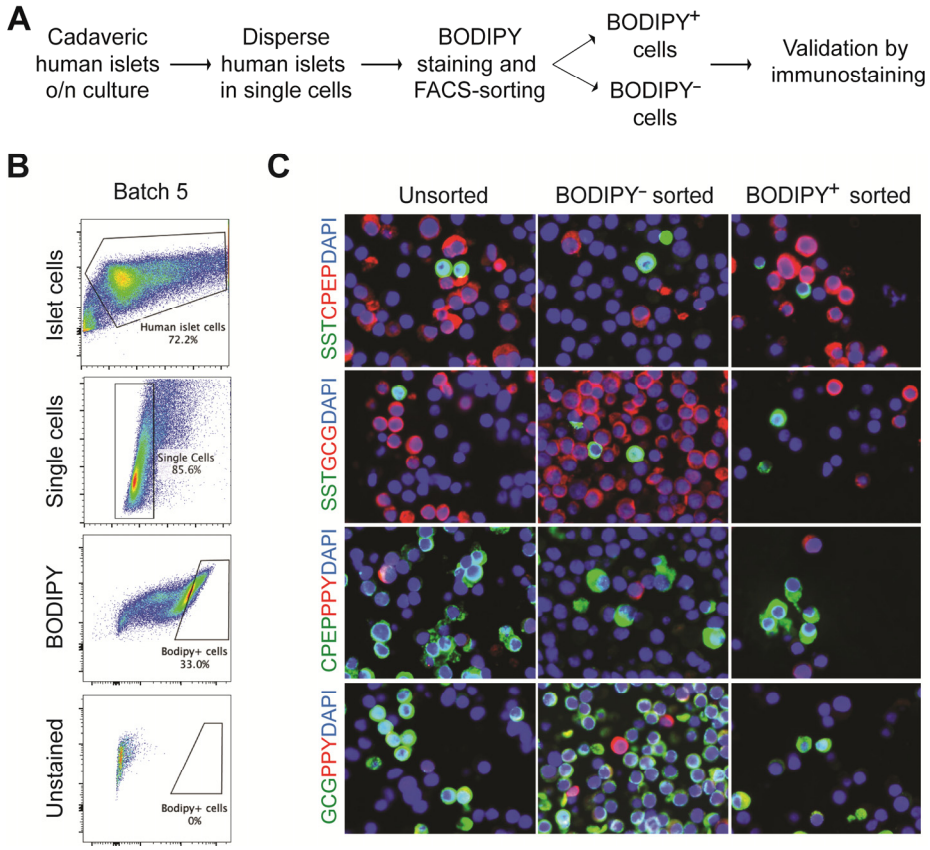


Figure S7. **(A)** Overview of BODIPY staining analysis and experimental approach for human pancreatic islets. **(B)** Representative flow plots show the sorting of human islets cells from a single donor. Cell debris was excluded by forwarding versus side scatter (FSC-A vs SSC-A) and singlets were identified by forward scatter area versus wide (FSC-A vs FSC-W). BODIPY stained cells were isolated according to unstained samples. **(C)** BODIPY^{+} and BODIPY^{-} human islet cells were collected after FACS. Cells were embedded in agarose and paraffin for sectioning. Slides were stained for islet hormones. Representative images show insulin (CPEP), glucagon (GCG), somatostatin (SST), pancreatic polypeptide (PPY) staining in unsorted, BODIPY^{+} and BODIPY^{-} cell populations. Nuclei stained with DAPI (blue).

Zn(II)-mediated hydrolysis of ZnPD5

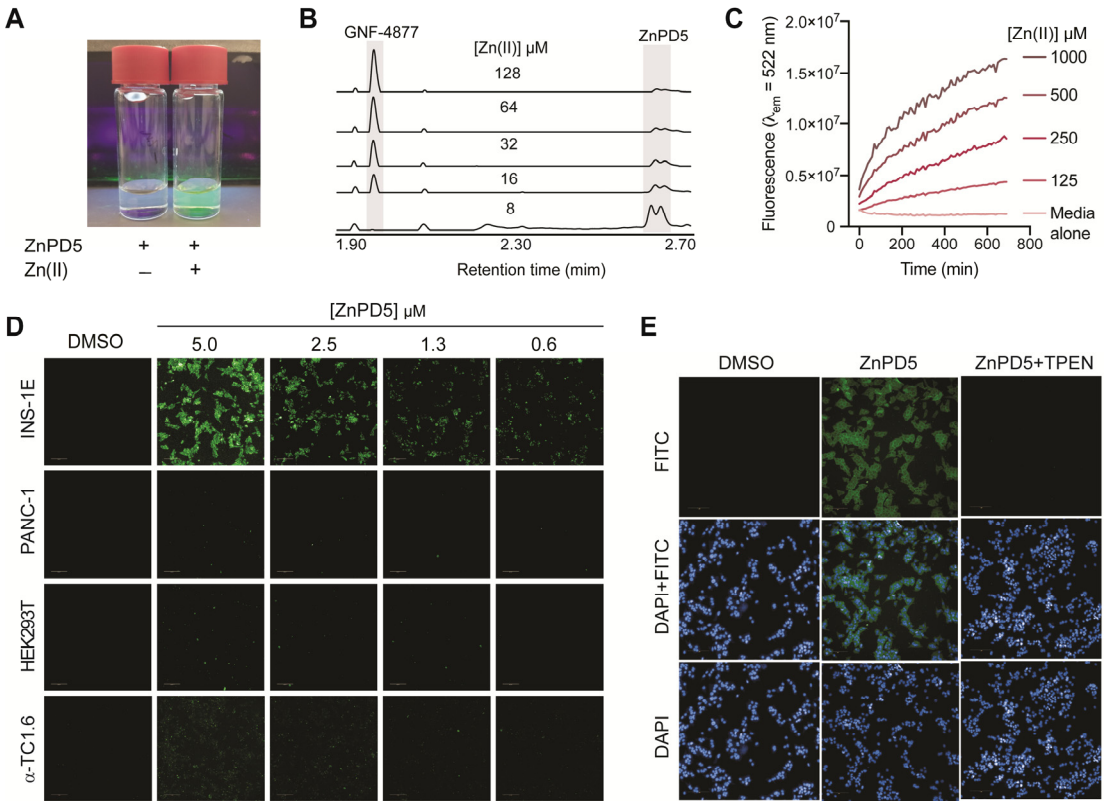


Figure S8. (A) The appearance of fluorescence signal after Zn(II)-mediated uncaging of ZnPD5. Images were captured under UV light (λ = 365 nm). **(B)** Kinetic profile of GNF-4877 (compound 3) release from ZnPD5 (10 μM) upon incubation with different amounts (8-128 μM) of Zn(II) in PBS. **(C)** Stability of ZnPD5 (10 μM) in INS1E cell culture medium alone and after addition of different amounts of Zn(II) (125-1000 μM) monitored by the fluorescence signal (λ_{ex} = 490nm, λ_{em} = 522 nm) of the released ZP1. **(D)** Representative images of the dose-dependent release of GNF-4877 (compound 3) from ZnPD5 in INS-1E cells in the FITC channel. **(E)** Fluorescence image showing inhibition of ZnPD5 (5 μM) staining of INS1E cells in the presence of a metal chelator (TPEN, 25 μM).

Zn(II)-mediated hydrolysis of ZnPD5 in Human Islet

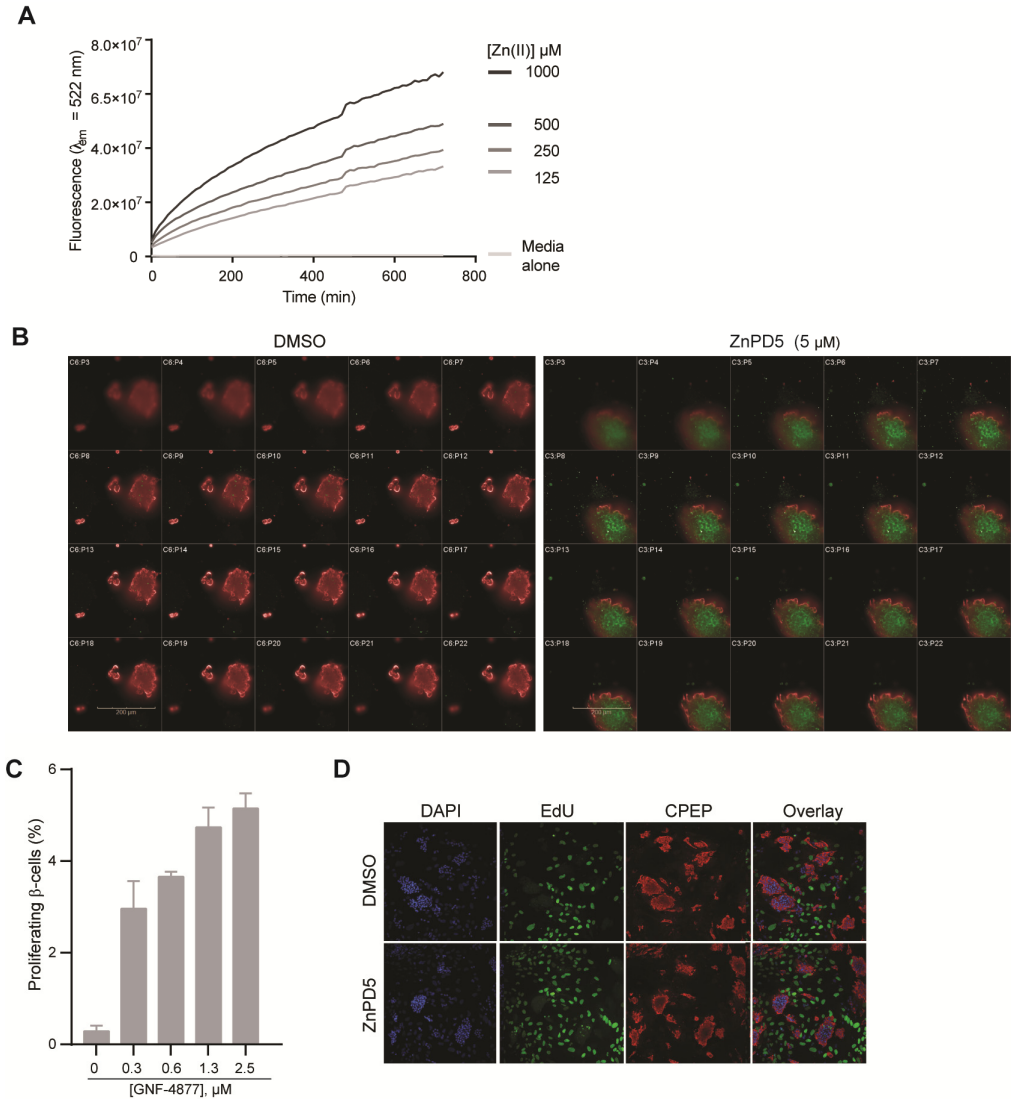


Figure S9. (A) Stability of ZnPD5 (10 μM) in human islet cell culture medium alone and after addition of different amount of Zn(II) (125-1000 μM) monitored by the fluorescence signal (λ_{ex} = 490nm, λ_{em} = 522 nm) of the released ZP1. **(B)** β-cell-selective staining of ZnPD5 in intact human islets. Undissociated human islets were incubated with either DMSO or ZnPD5 (5 μM) for 3 h in culture medium in the incubator before processing for immunostaining and confocal fluorescence microscopy. Islets were imaged at 18 different planes with 1 μm spacing. Anti-C-peptide antibody (red) was used as the β-cell counterstaining and the FITC channel represents the dissociated ZnPD5. The co-localization of FITC and red signal indicates the β-cell-selective release of ZnPD5. **(C)** Proliferation profile of human islet β-cells after incubating with GNF-4877 (0–2.5 μM) for 6 days. **(D)** Representative images for proliferative human islet cells as probed with EdU assay. Cells were cultured in the presence of EdU (10 μM) and ZnPD5 (2.5 μM) for 6 days. Green and red channels represent EdU and anti-C-Peptide staining, respectively.

^{1}H and ^{13}C NMR spectra of ZnPDs

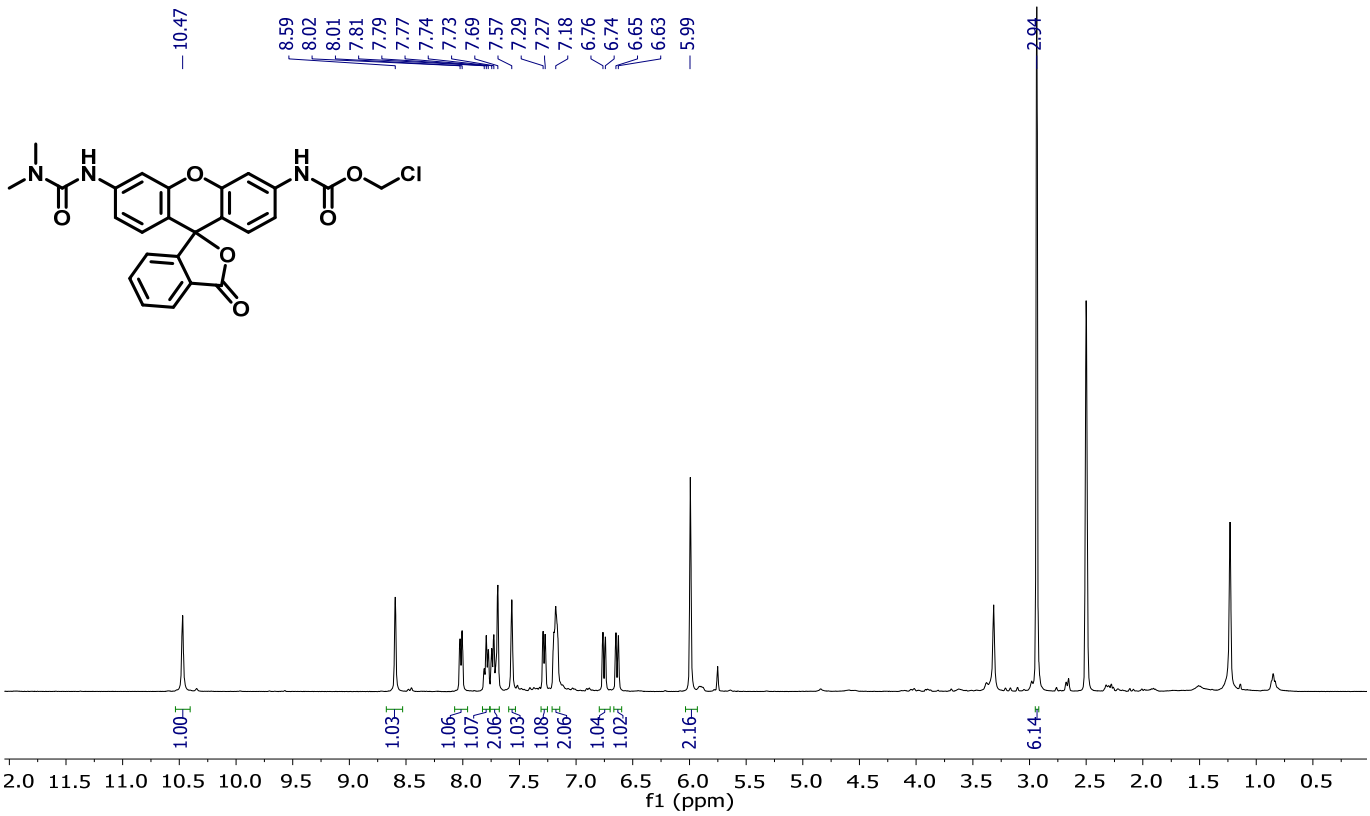


Figure S10. ^{1}H NMR spectrum of compound 1.

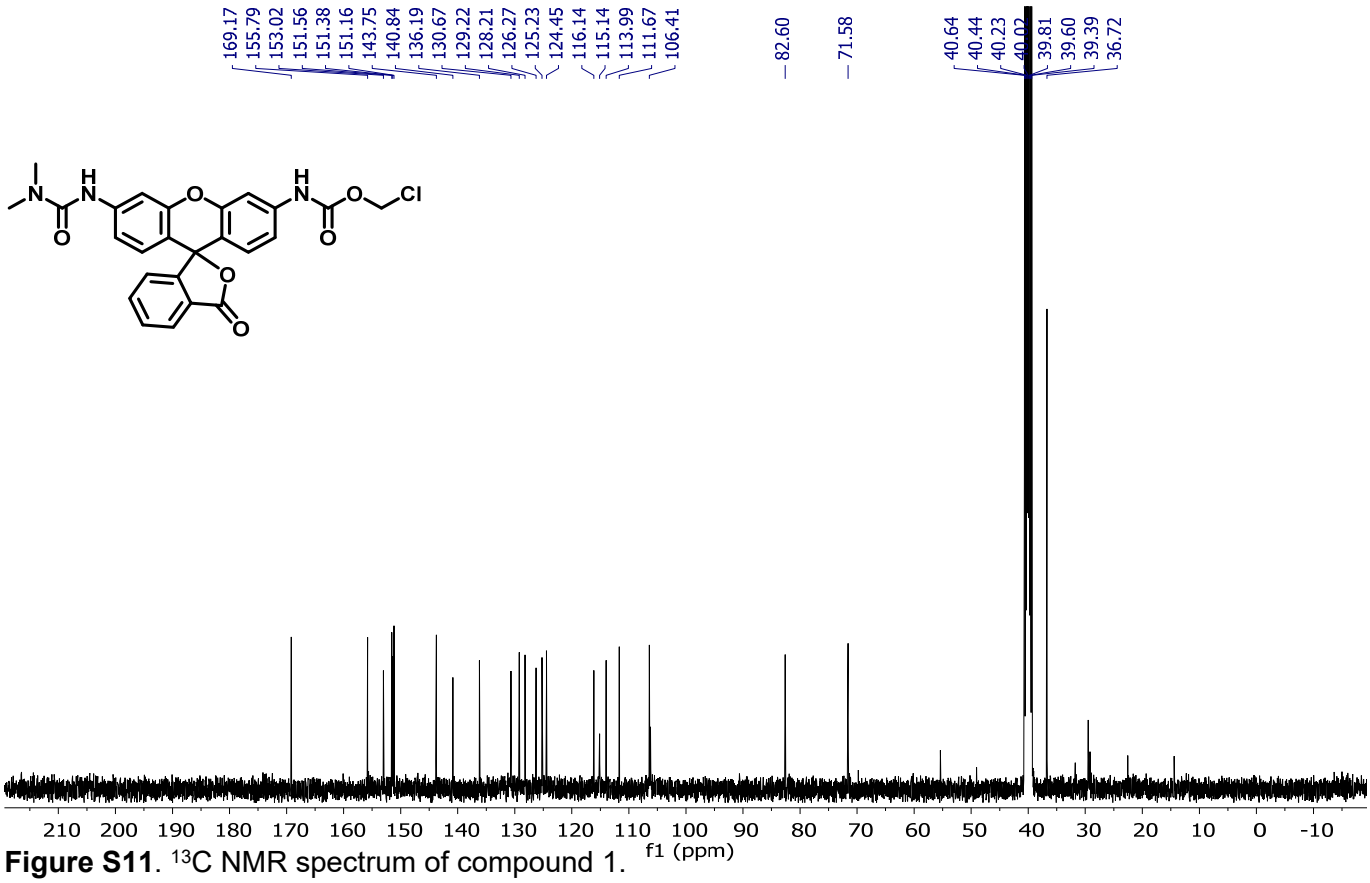


Figure S11. ^{13}C NMR spectrum of compound 1.

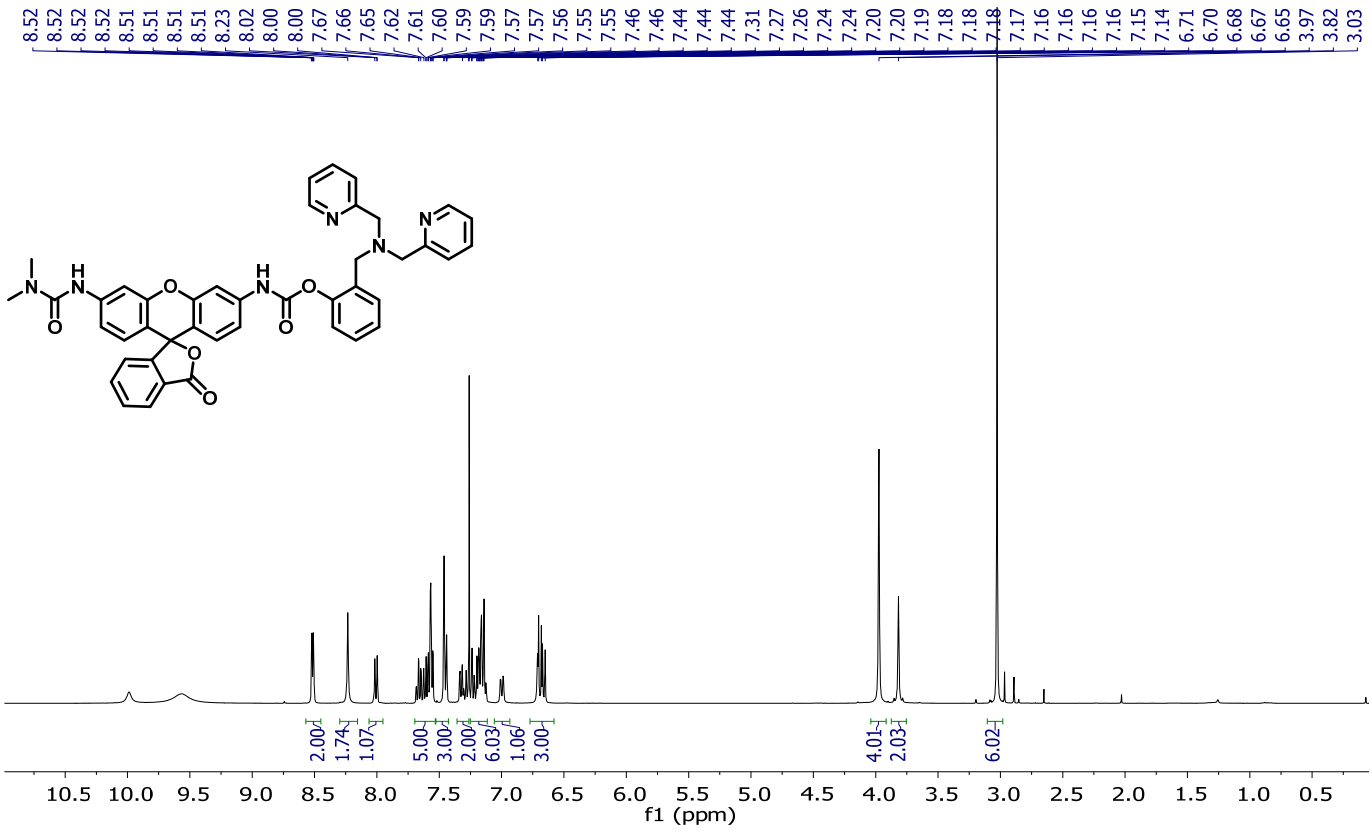


Figure S12. ^{1}H NMR spectrum of ZnPD1.

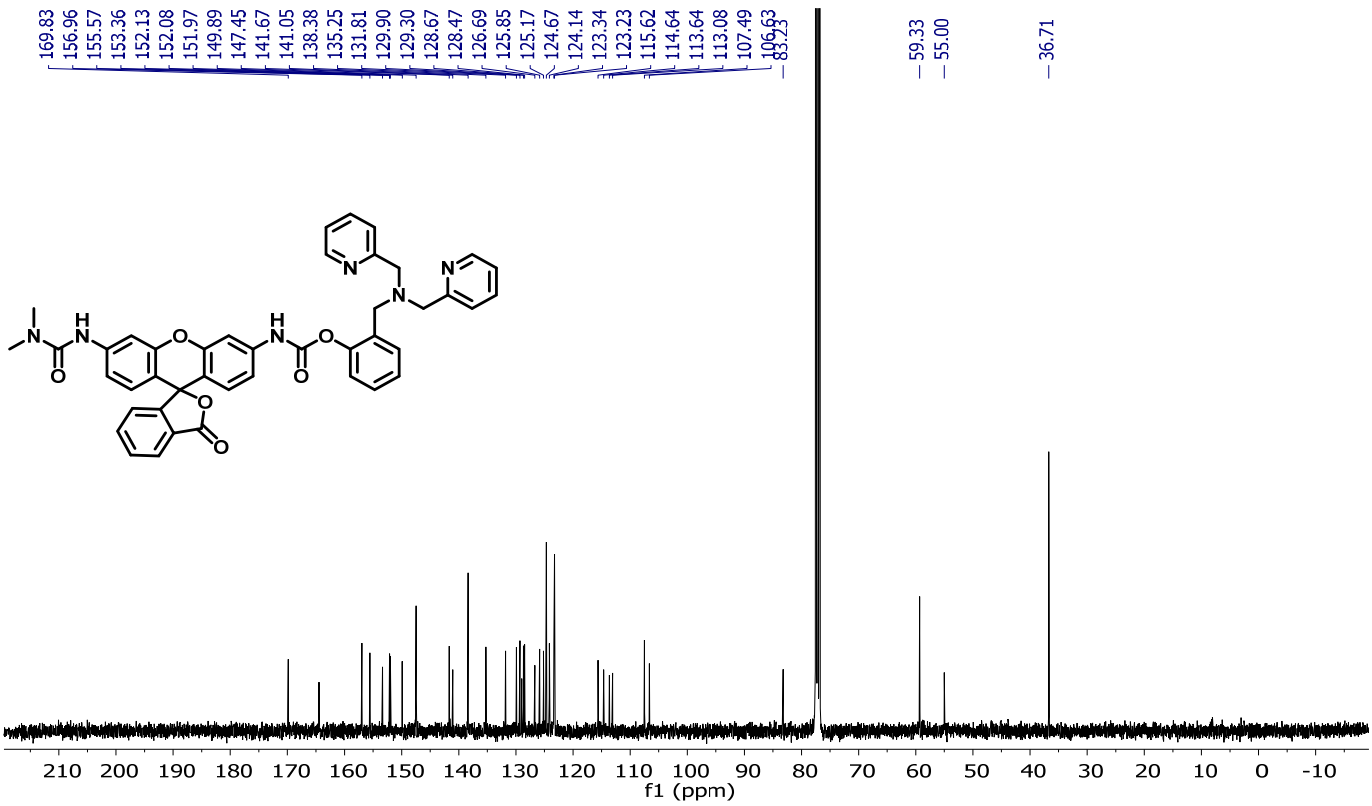


Figure S13. ^{13}C NMR spectrum of ZnPD1.

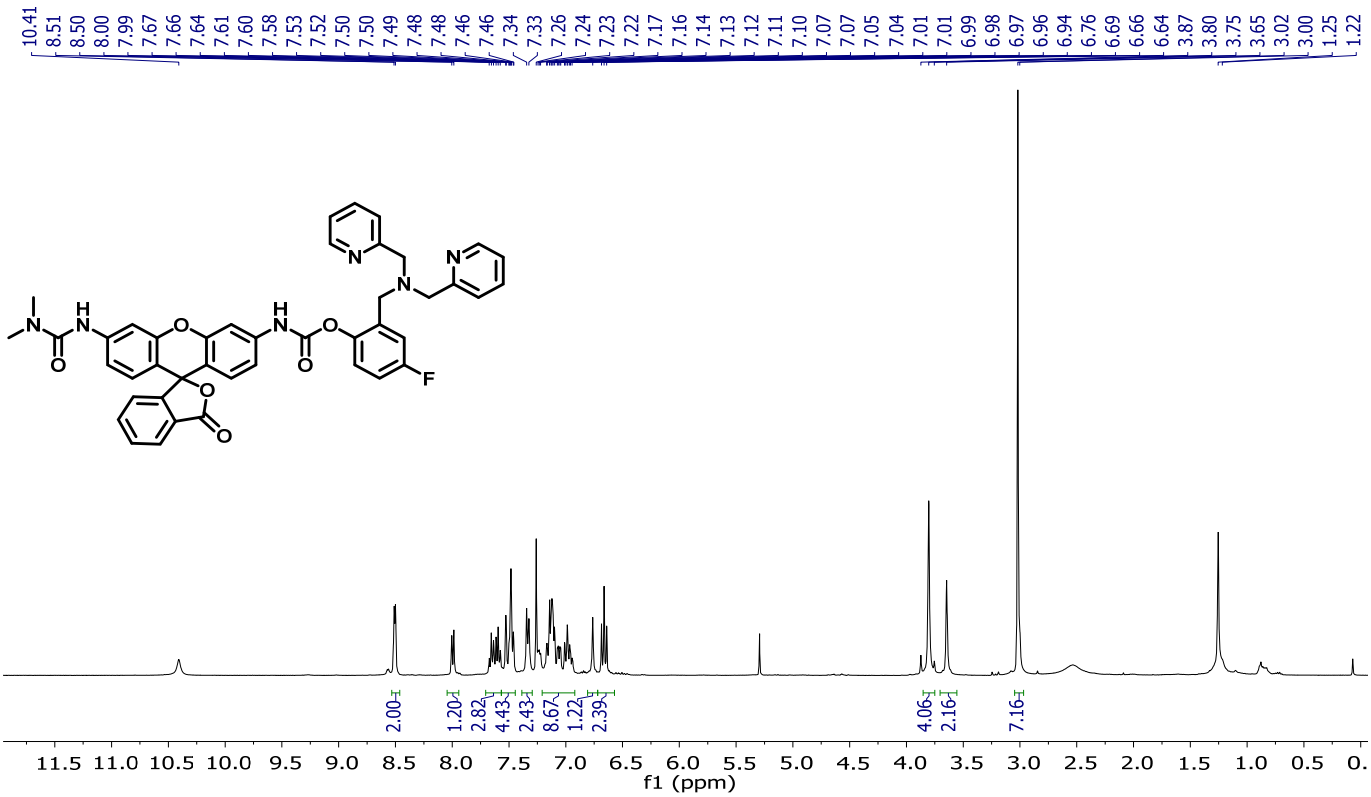


Figure S14. ^{1}H NMR spectrum of ZnPD3.

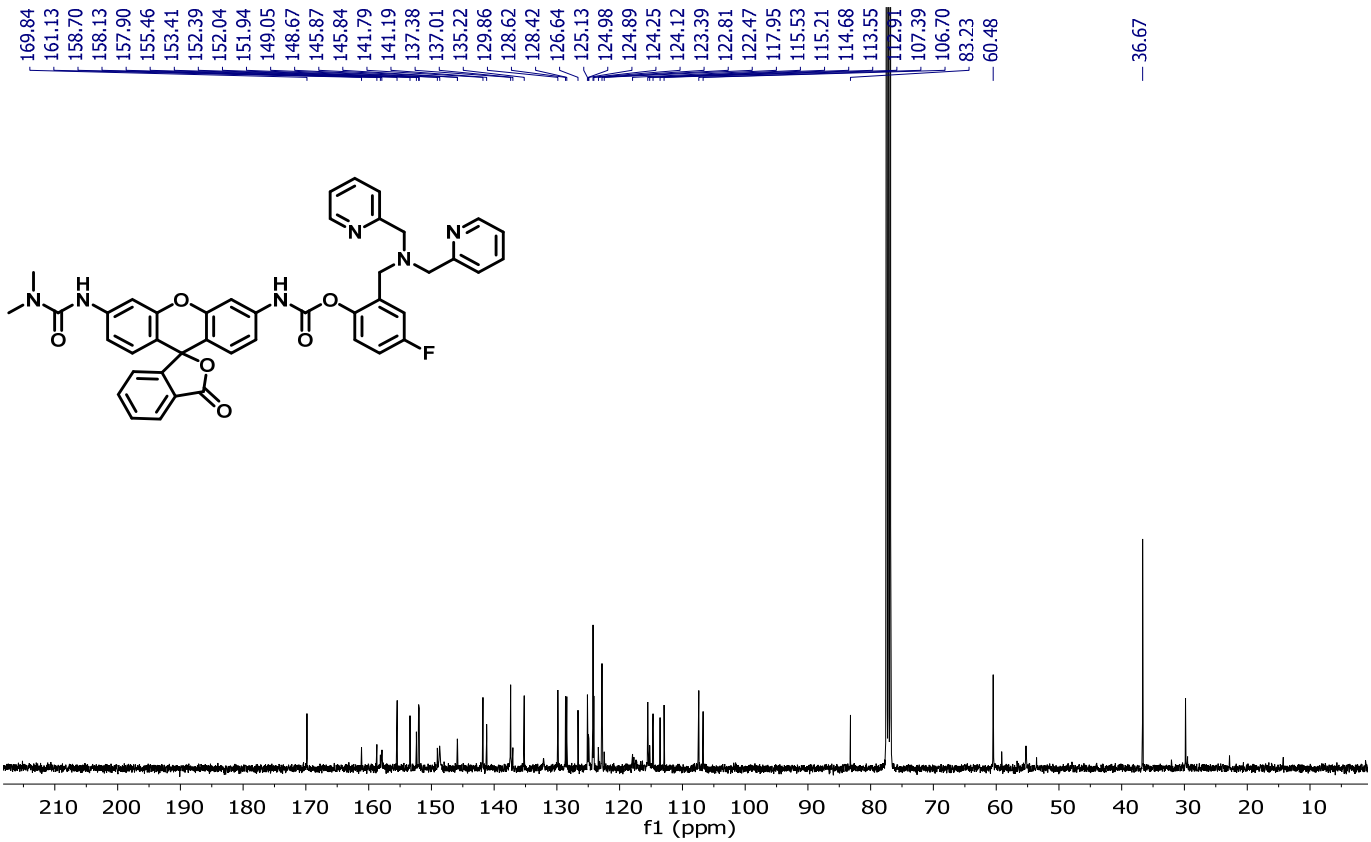


Figure S15. ^{13}C NMR spectrum of ZnPD3.

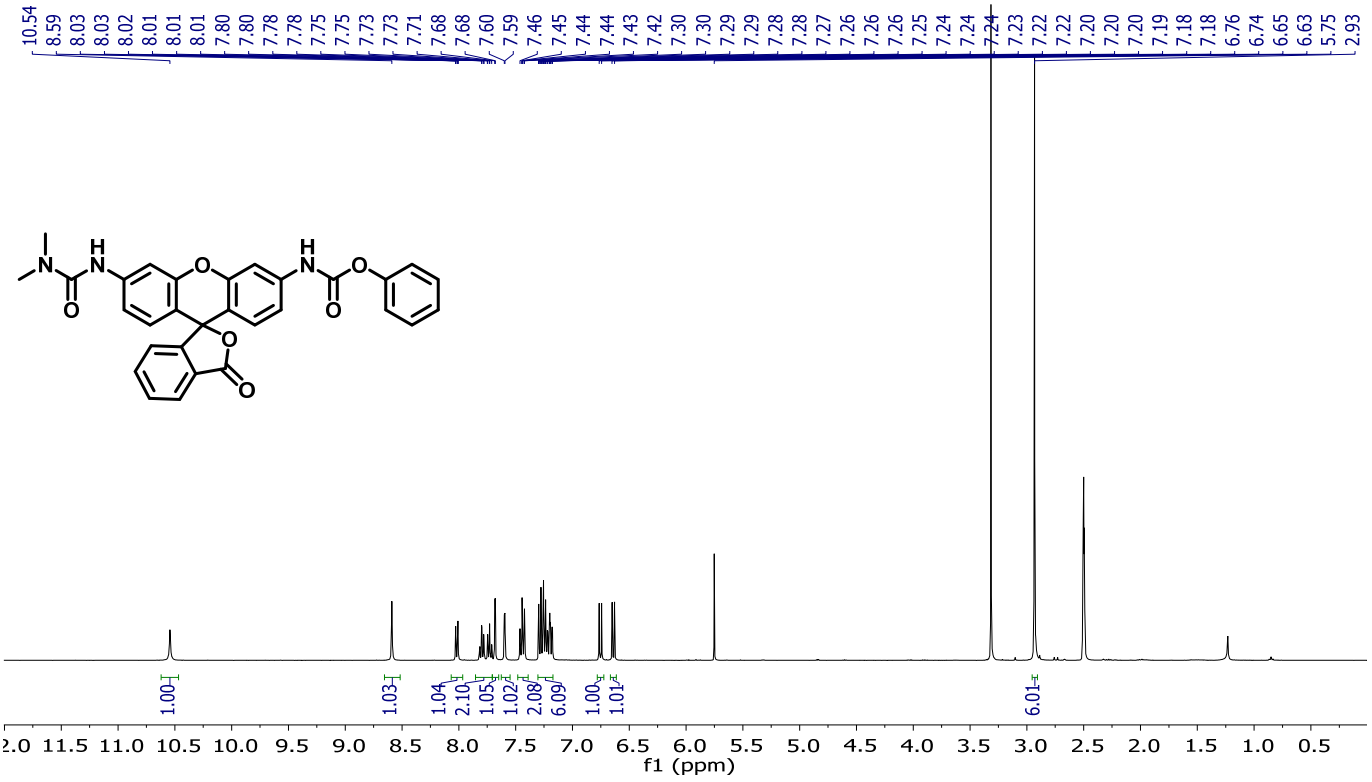


Figure S16. ^{1}H NMR spectrum of ZnPD1ctrl.

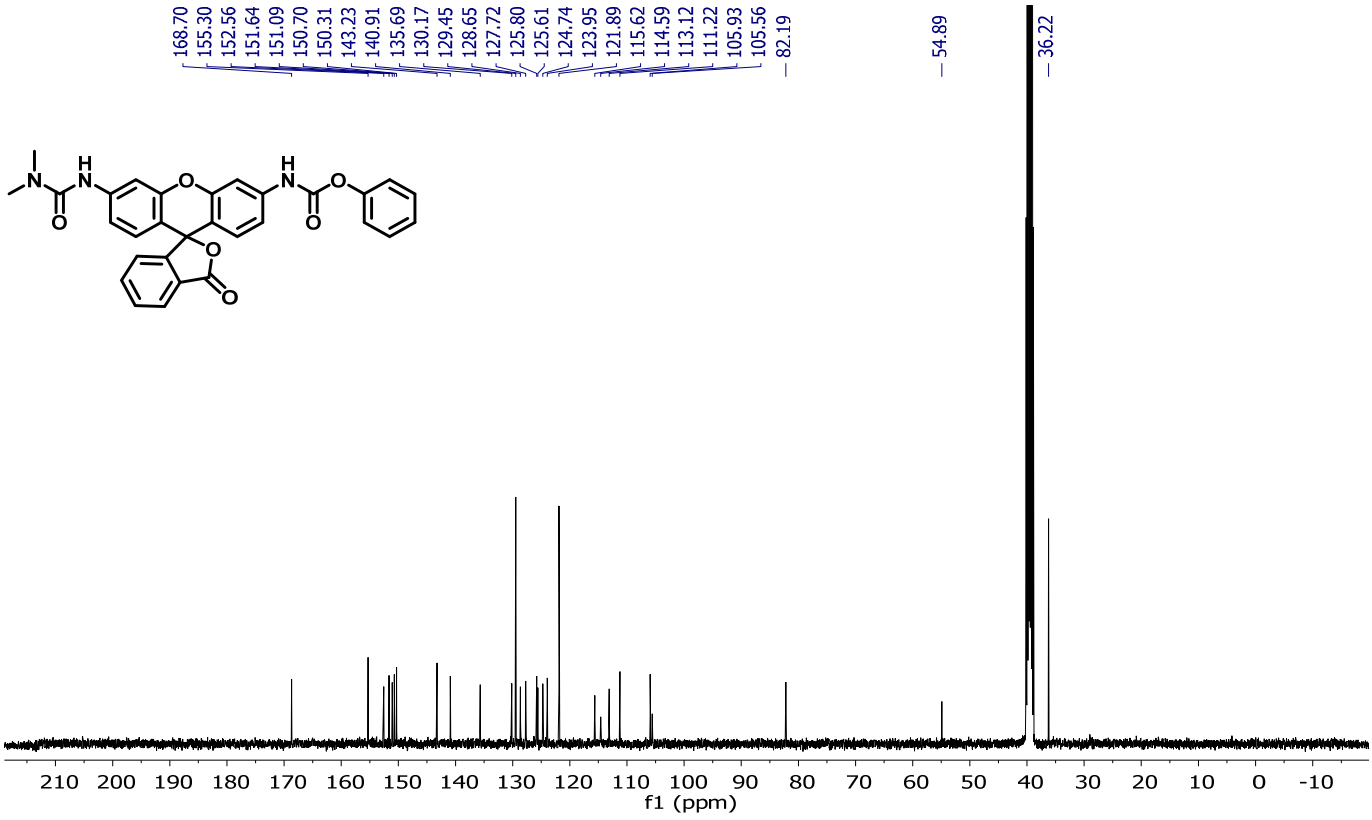


Figure S17. ^{13}C NMR spectrum of ZnPD1ctrl.

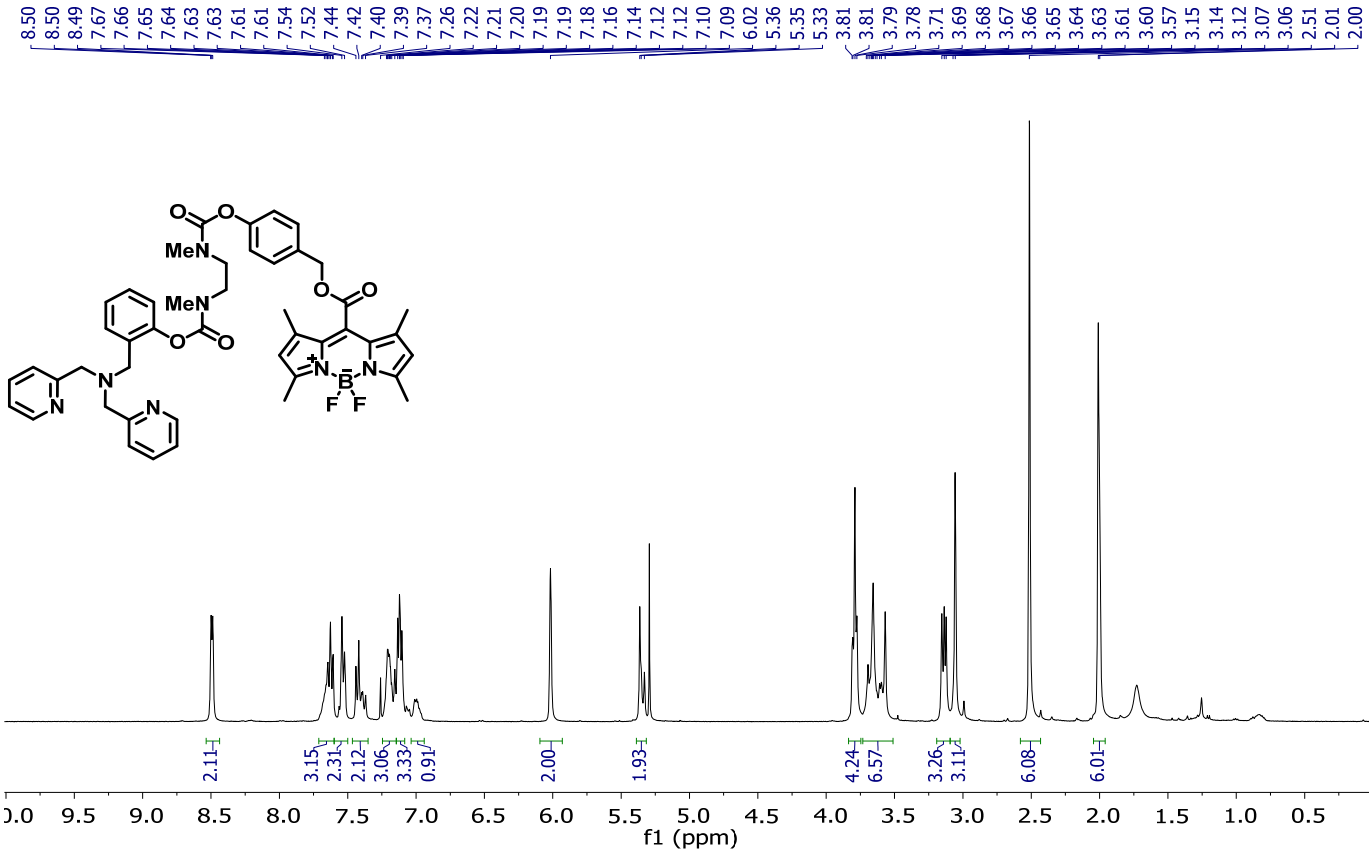


Figure S18. ^{1}H NMR spectrum of ZnPD4.

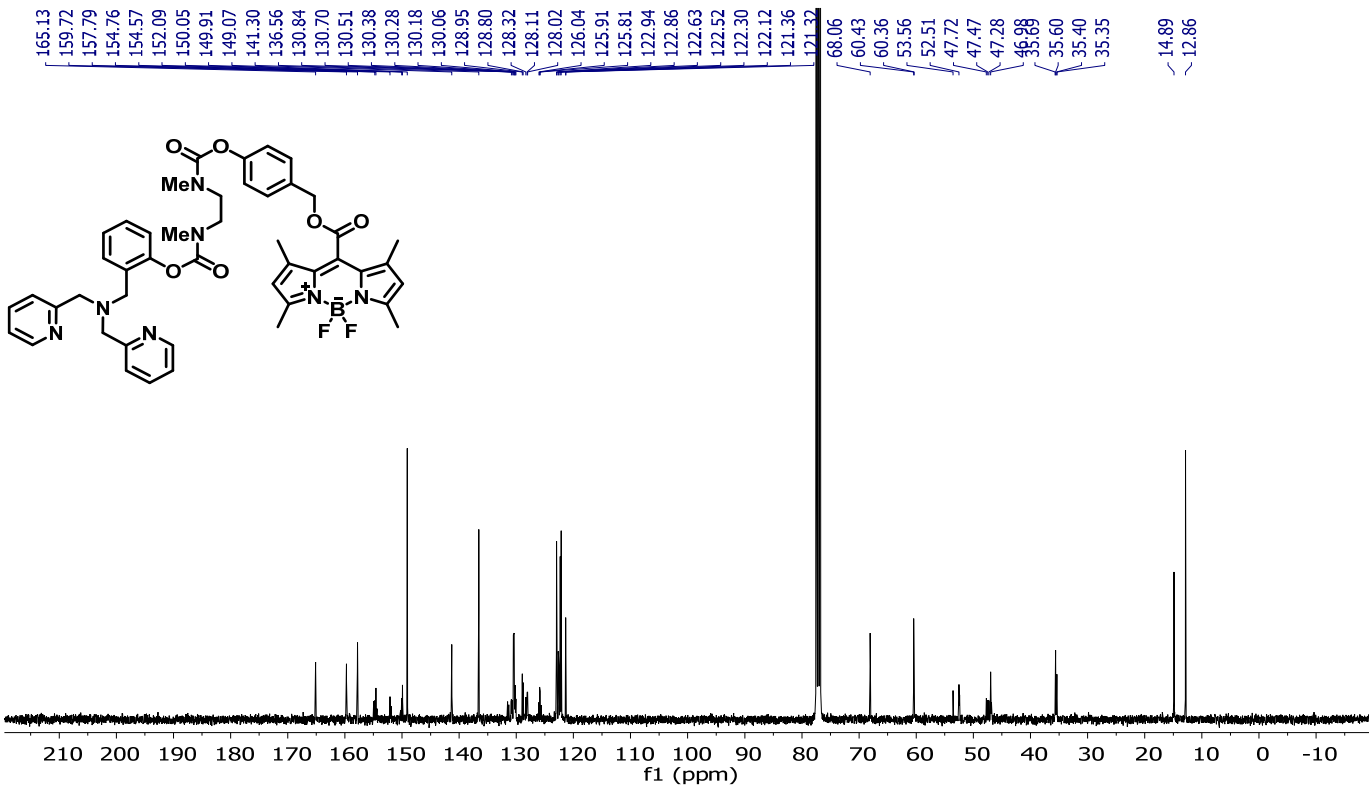


Figure S19. ^{13}C NMR spectrum of ZnPD4.

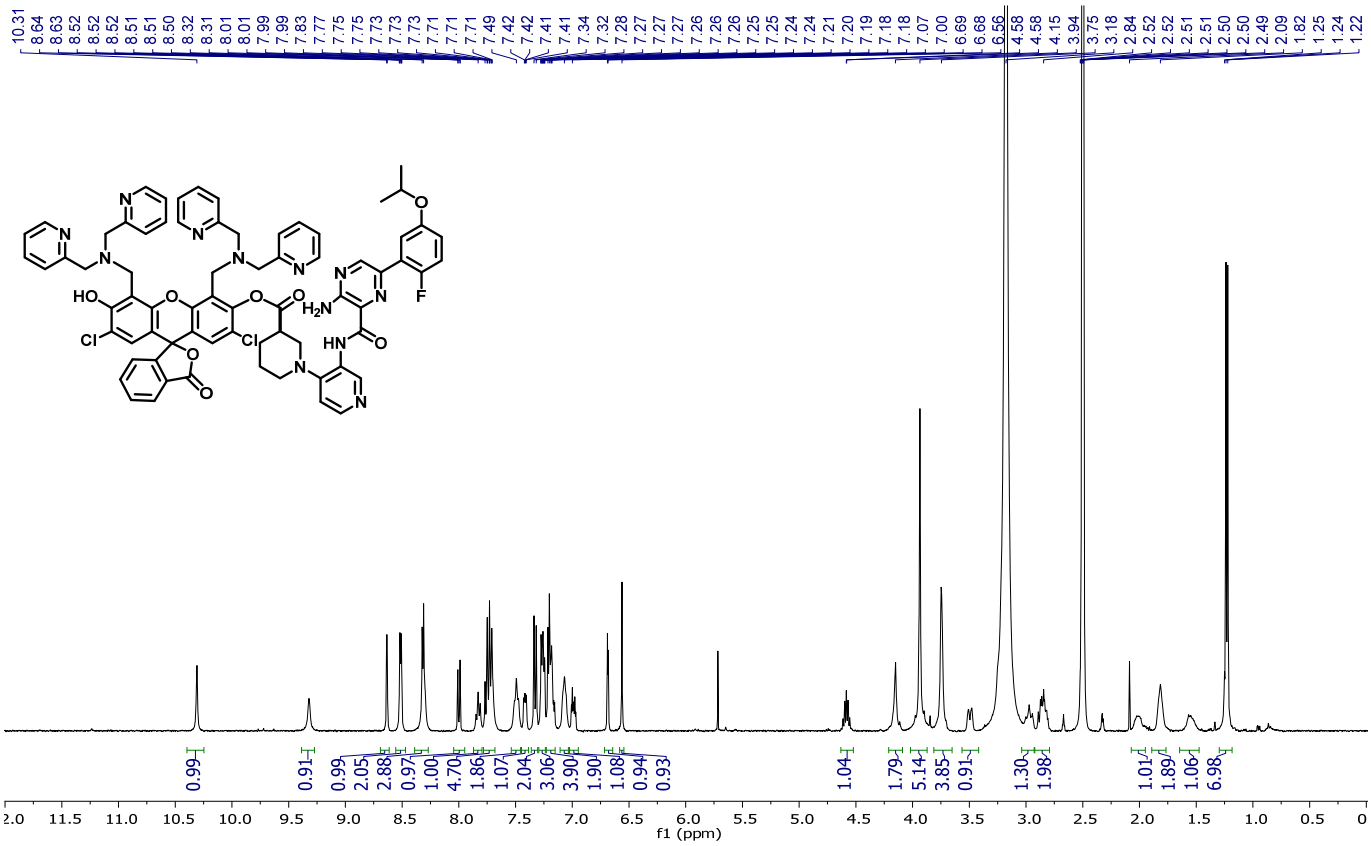


Figure S20. ^{1}H NMR spectrum of ZnPD5.

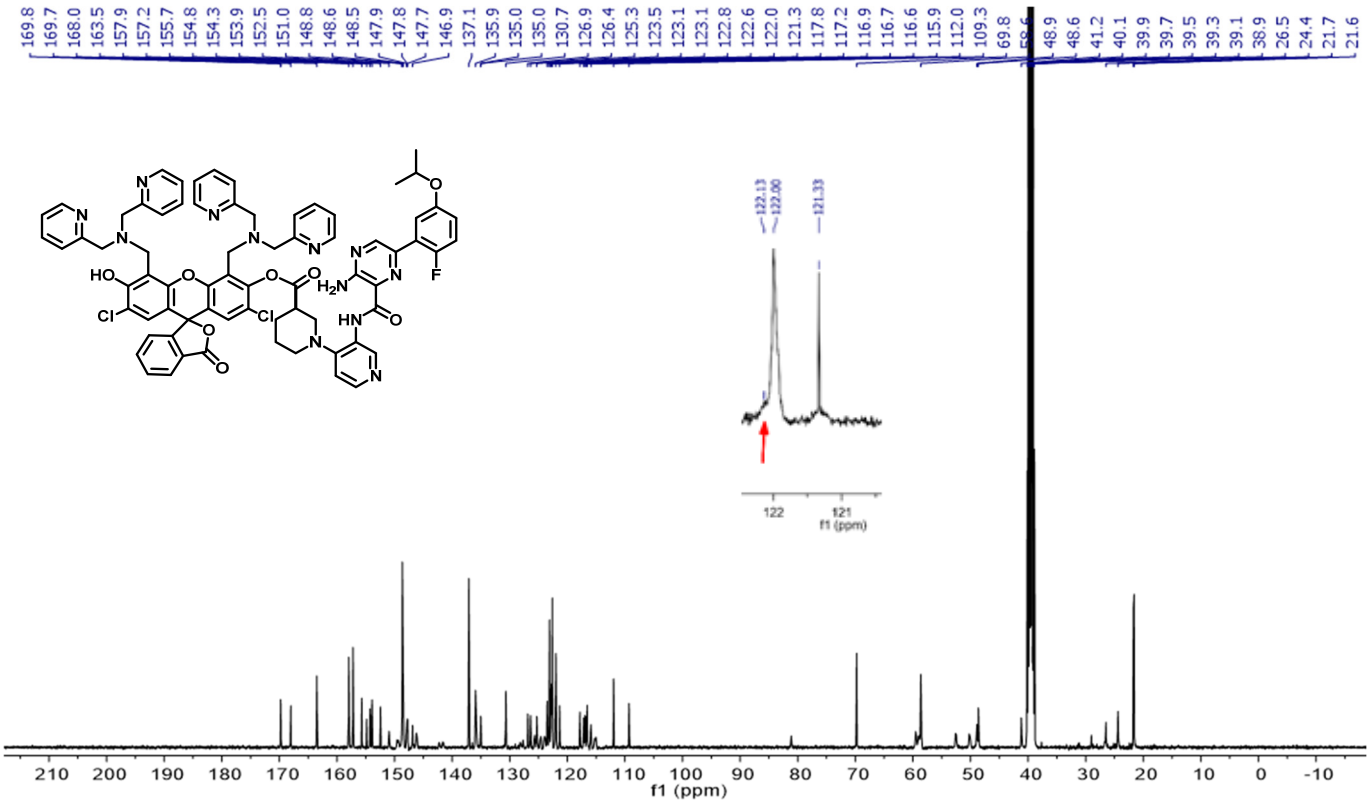


Figure S21. ^{13}C NMR spectrum of ZnPD5.

ZnPD5 LCMS Trace

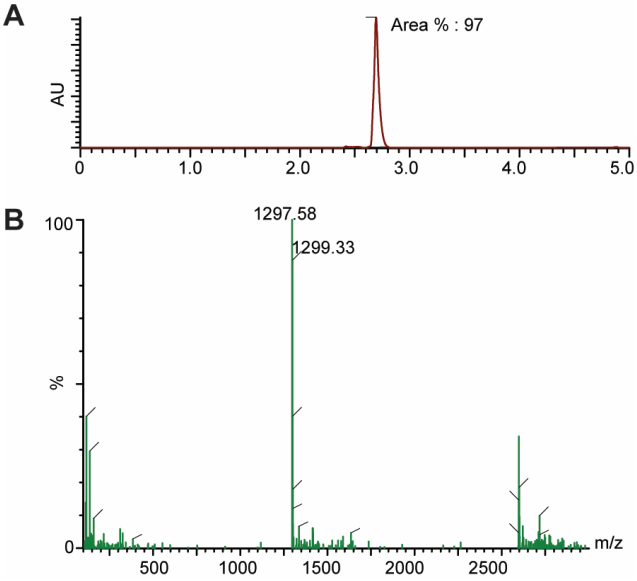


Figure S22. (A) LCMS UV (254 nm) trace for ZnPD5 **(B)** Observed mass of ZnPD5.