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Supporting Information

Chemically Robust Fluoroalkyl Phthalocyanine-Oligonucleotide Bioconjugates and their GRP78 Oncogene Photocleavage Activity

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EXPERIMENTAL PROCEDURES

Materials and Methods. All commercial reagents and solvents (ACS grade) were used without further purification, unless stated otherwise. Cesium fluoride was dried *in vacuo* for 2 days over P_4O_{10} at 100 °C prior to use. Acetonitrile (MeCN) used for the synthesis of the perfluoro-(4,5-diisopropyl)phthalonitrile precursor was freshly distilled from calcium hydride before use. Perfluoro-(4,5-diisopropyl)phthalonitrile,[24] **1** and 4-(3,4-dicyanophenoxy)benzoic acid,[25] **2** were prepared according to a published literature procedure. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Chromatographic separations were carried out on silica gel 60 (63–200 μm). Thin-layer chromatography was performed on pre-coated silica gel 60 F_{254} plates.

^1H, ^{13}C and ^{19}F NMR spectra were obtained at ambient temperature on a Varian 500 MHz NMR spectrophotometer. ^1H and ^{13}C NMR spectra were referenced internally to residual solvent peaks and ^{19}F chemical shifts were referenced to an internal $CFCl_3$ (δ = 0.00) standard. NMR spectral data were processed using the ACD (version 12.01) software. Infrared spectra were obtained on a Nicolet FT-IR spectrophotometer. Electronic absorption spectra were collected using a Perkin Elmer UV-Vis spectrophotometer. High-resolution mass spectra (HRMS) were acquired at the University of Michigan (Ann Arbor, MI) by electron spray ionization in negative mode. A microwave CEM Discovery microwave reactor was used for Pc synthesis. Reactions were performed using a septum-sealed reaction vessel (10 mL) which included a thick-walled Pyrex glass reaction vessel sealed with Teflon septa caps. Melting points were taken using capillary tubes under air in a Mel-Temp apparatus and are uncorrected.

Synthesis of $F_{48}H_7$(COOH)PcZn, 3. 3,6-difluoro-4,5-bis(perfluoropropan-2-yl) benzene-1,2-dinitrile **1**, (100 mg, 0.199 mmol), 4-(3,4-dicyanophenoxy)benzoic acid, **2**, (53 mg,

0.199 mmol), zinc(II) acetate (18.3 mg, 0.999 mmol) and catalytic amounts of ammonium molibdate were mixed with few drops of nitrobenzene in a glass vial, sealed with a Teflon cap and heated in the microwave reactor at 190 °C for 20 minutes. The crude product was purified by column chromatography on silica gel using 4:6 v/v hexane/acetone. F_{64}PcZn was isolated as a green-blue solid (31% yield) while the desired product, $F_{48}H_7$(COOH)PcZn, **3**, was isolated as a green solid. The latter was further purified by chromatography using 1:9 v/v of MeCN (contain 0.1 % TFA)/Hexane. Yield: 25.3 mg, 20.8%. ^{1}H NMR (500 MHz, Acetone-d_6): δ 7.13 - 7.31 (m, 2 H), 7.46 - 7.59 (m, 1 H), 7.62 - 7.78 (m, 1 H), 7.80 - 8.20 (m, 3 H). ^{19}F NMR (470 MHz, Acetone-d_6): δ -164.57 (br. s., 6 F), -104.23 (br. s., 6 F), -72.87- -70.37 (m, 36F). IR (NaCl, cm^{-1}): 2957, 2924, 2853, 1726, 1591, 1462, 1379, 1274, 1122, 1074, 951, 801. HRMS (-ve ESI) *m/z* ($[M+Cl]^-$) calcd: 1862.8938; obsd: 1862.8964. UV-Vis ($CHCl_3$) λ_{max} (log ε) 710 (5.02), 672 (4.96), 608 (4.32), 378 (4.68) nm (L mol^{-1} cm^{-1}).

Photooxidation of *β*-citronellol. The catalyst $F_{48}H_7$(COOH)PcZn, **3**, was dried to constant weight at 150 °C prior to use. Photooxidation were performed at 25±0.2 °C under oxygen atmosphere (99.998% purity) in a closed, 100 mL double-walled jacketed glass vessel, while stirring at 180 rpm for 4 hrs. The temperature was kept constant with the use of a Lauda Brinkmann Ecoline RS-106 refrigerating water bath circulator. Oxygen was dosed with a Dosimat 665 dispenser (Metrohm, Switzerland) and its consumption was measured with a manometer in a closed system (see Figure S6). Catalyst stability measurements were performed by UV-Vis absorption measurements (300-800 nm) of aliquots (1 mL, 0-4 h) extracted periodically from the reaction mixture. The photooxidation reaction mixture consisted of Pc, **3** (20±2 μM) catalyst dissolved in ethanol (50 mL) with (*S*)-(–)-citronellol (180 μL, 1.0 mmol). Illumination was performed using the 300 W halogen lamp of a Kodak Ektagraphic III slide

projector, creating a light intensity of 2.5±0.1 × 10^5 lux, measured at the outer wall of the reaction vessel with an EA33 model light meter (Extech Instruments, Waltham, MA).

Solid-Phase Oligonucleotide Synthesis. Synthesis of alkyl (C_6) amino linker GRP78 antisense oligonucleotide, **9**, (5'-CTTCATCTTGCCGGCGCT-3'), sense oligonucleotide, **7**, (5'-AGCGCCGGCAAGATGAAG-3') and sense RNA oligonucleotide, **8**, (5'-AGCGCCGGCAAGAUGAAG-3') sequences were performed on an Unylinker controlled pore glass (CPG) support (Chemgenes Co.) using a 1 μmol scale automated synthesis cycle on a ABI 3400 DNA synthesizer. All phosphoramidites were dissolved in anhydrous MeCN yielding 0.1 M solutions. The coupling times were 20 sec using 0.25 M 5-ethylthiotetrazole (ETT) in MeCN as activator. The detritylation times were set to 2 min using a solution of 3% dichloroacetic acid in CH_2Cl_2 (DCM). Capping and oxidation steps were performed using a mixture of acetic anhydride/*N*-methyl imidazole in MeCN and a solution of 0.01 M iodine in pyr/THF/H_2O, respectively. Following synthesis, oligonucleotides were cleaved from the CPG and deprotected with NH_4OH:EtOH (3:1 v/v) for 16 hrs at 55 ºC. The crude oligonucleotides were evaporated to dryness, extracted from the CPG with autoclaved H_2O (1 mL) and quantitated by UV/Vis spectrophotometry. Crude oligonucleotides were purified by reverse-phase ion-pairing HPLC on a Waters 2695 Alliance system equipped with a Nova-pak C-18 reverse phase column (3.9 x 150 mm, 4 μm particle size) and gradient elution method of 5-95% A over 23 min (A: 20% MeCN in 0.1 M TEAA). The molecular masses of pure oligonucleotides were confirmed by ESI-MS. In the case of RNA, **8**, CPG-bound RNA was transferred into autoclaved eppendorf tubes and treated with a 1 mL solution of 1:1 v/v ammonium hydroxide/methylamine (AMA) and heated at 65 °C for 10 min. AMA was evaporated *in-vacuo* and the CPG was washed twice with autoclaved water (500 μL). Crude RNA oligonucleotides were re-suspended in a mixture of 1:1

v/v DMSO:triethylamine trihydrofluoride (125 μL) to complete the 2'-desilylation reaction at 65 °C for 90 min. The crude RNA was precipitated from the reaction mixture with 3 M NaOAc (25 μL) in n-BuOH (1 mL). Precipitation was completed on dry ice for 2 hrs prior to centrifugation (12,000 rpm) leaving the crude oligonucleotides as a solid white pellet. Crude oligonucleotides were re-suspended in autoclaved water (1 mL) and the yields were determined by UV absorbance measurements at 260 nm. Crude RNA was purified by reverse-phase ion-pairing HPLC and characterized by ESI-mass spectrometry as previously described.

Preparation of the flouro-Pc antisense oligonucleotide bioconjugate, 6. The bioconjugate was produced on solid-phase, by combining the carboxy-derived perflouro phthalocyanine, **3**, (3 eq, 5 mg) with the GRP78 antisense **10**, oligonucleotide-bound resin (1 μmol). A coupling reagent (HCTU, 1.2 mg, 3 eq) was added, the mixture was suspended in DMF (1 mL) and a base (NMM, 0.66 μL, 6 eq) was added to initiate the reaction. The reaction was performed overnight (16 hrs) at room temperature, in an overhead shaker. The mixture was filtered, and the resin was washed successively with DMF (3 x 10 mL), MeCN (3 x 10 mL), THF (3 x 10 mL) and DCM (3 x 10 mL) and dried *in vacuo*. The fluoro-Pc oligoncucleotide-bound resin was cleaved and deprotected using NH_4OH:EtOH (1 mL, 3:1 v/v) for 24 hrs at 55 °C. The solution was centrifuged and concentrated to a solid pellet. The crude oligonucleotide was extracted in autoclaved water (1 mL), quanitated by UV-Vis spectrophotometry (Crude OD: 29, Absorption coefficient (ε): 159,100, Path-length: 1 cm, Concentration: 182 μM)* analyzed and purified by ion pairing reverse-phase HPLC as described above. The desired fluoro-Pc oligonucleotide bioconjugate, **6**, was isolated in purity >95% and 18% yield (1.35 mg) characterized by ESI mass spectrometry (See Table S1) and quanitated by UV-Vis

spectrophotometry (Pure OD: 3, Absorption coefficient (ε): 159,100, Path-length: 1 cm, Concentration: 19 μM)*

* Absorption coefficient (ε) was calculated using Intergrated DNA Technologies' OligoAnalyzer 3.1. Optical Density (OD) was converted to Concentration using converter.

UV-Vis Spectroscopy. Optical absorption spectra were acquired with a Perkin Elmer or a Varian Cary 300 dual beam UV-Vis spectrophotometer. Absorption values were obtained for **3** (10 μM) in chloroform/ethanol or for oligonucleotides **6-10** (0.67 μM) in a physiologically relevant phosphate buffer (140 mM KCl, 5 mM NaH_2PO_4, 1 mM $MgCl_2$, pH = 7.2). Absorption measurements were recorded at 300-800 nm for **3**, **6** and 260 nm for oligonucleotides **6-10**, which were quantified according to the Beer-Lambert law. The data was averaged over a range of five different absorbance scans and reported as a mean value with a standard deviation no greater than 0.1 a.u.

Electron spray ionozation mass spectrometry. All oligonucleotide mas spectra were acquired on an HTCS LCMS system at Novatia, LLC for 100 pmol of oligonucleotide sample dissolved in 1 mL H_2O and directly injected for ESI-MS. The data was processed by a ProMass software (see supporting info, Table S1). For high-resolution mass spectrometry of Pc, **3**, the sample was analyzed by direct injection on TOF/Q-TOF Mass Spectrometer at University of Michigan, Mass Spectrometry service laboratory.

Thermal denaturation experiments, T_m. Thermal denaturation studies were conducted on a Varian UV-Vis Cary 300 dual beam spectrophotometer equipped with a termperature controller. Complementary DNA and RNA oligonucleotides were annealed in a physiologically

relevant phosphate buffer (140 mM KCl, 5 mM NaH_2PO_4, 1 mM $MgCl_2$, pH = 7.2). The solutions were denatured at 95 ºC for 1 min then cooled to room temperature over 2.5 h and kept at 4 ºC overnight prior to conducting experiementation. The thermal melts were run from 5-90 ºC with temperature gradient increments of 0.5 ºC/min and data points collected every 0.5 ºC/min at 260 nm. The T_m measurements were calculated according to the temperature at which 50% of the duplex denatured to single strands from a plot comparing the changes in hyperchromicity *vs* temperature.

Photooxidation and chemical cleavage assay. The Pc labeled GRP78 antisense oligonucleotide, **6**, (6.7 μM) was annealed with a complementary DNA or RNA sequences (**7-9**, 6.7 μM) in a physiological phosphate binding buffer (140 mM KCl, 1 mM $MgCl_2$, 5 mM Na_2PHO_4 adjusted to pH 7.2). The unlabeled native GRP78 oligonucleotide sequences, **7-9**, were also hybridized and used as controls. Air was passed inside the microtube reactor (1 mL) to saturate the solution with oxygen. The reaction was initiated by shining light (>250000 Lux) onto the samples at room temperature (22 ^oC). Aliquots (7 μL), collected at different time points (0-12 h), were transferred to separately sealed microtubes and stored in the absence of light at 4 °C until further use. Control experiments were also conducted as previously described in the absence of oxygen and/or light. Following the completion of the photooxidation reaction, the aliquots were dissolved in 1 M piperidine in water (150 μL, pH 12) and incubated for 45 min at 95° C. The samples were centrifuged and concentrated to dryness on a Savant speedvac concentrator. Samples were then re-suspended in 80% formamide in autoclaved water (10 μL) and loaded on a 24% denaturing (7 M urea) PAGE. Following electrophoresis gels were visualized under short-UV shadowing (265 nm) and subsequently placed in a Stains-All®

(Sigma) dye solution (25 mg Stains-All®, 50 mL isopropyl alcohol, 25 mL formamide, 125 mL water) for visualizing the resolved bands.

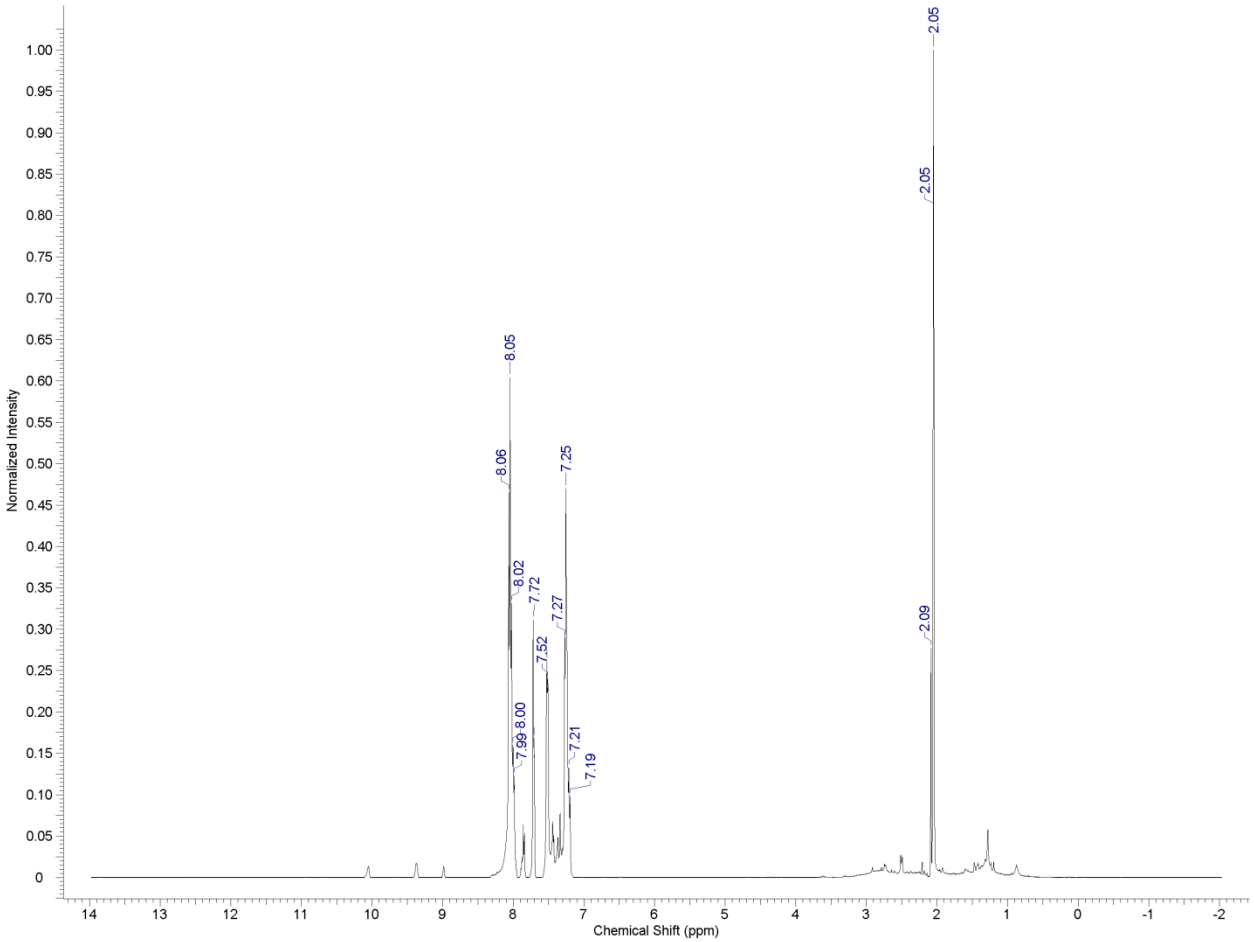

Figure S1. ^{1}H NMR spectrum of $F_{48}H_{7}$(COOH)PcZn, **3**, in $(CD_3)_2CO$.

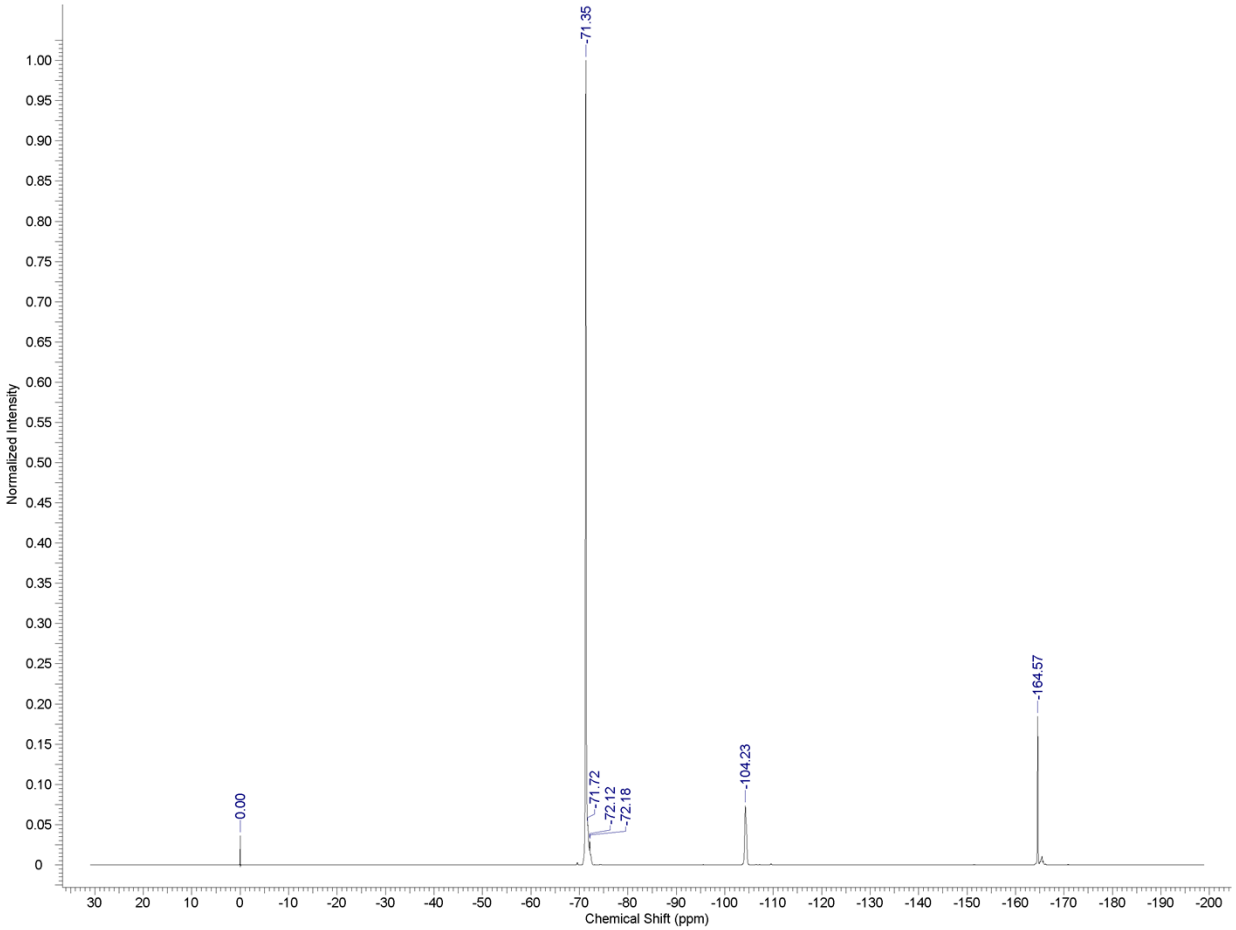

Figure S2. ^{19}F NMR spectrum of $F_{48}H_7(COOH)PcZn$, **3**, in $(CD_3)_2CO$.

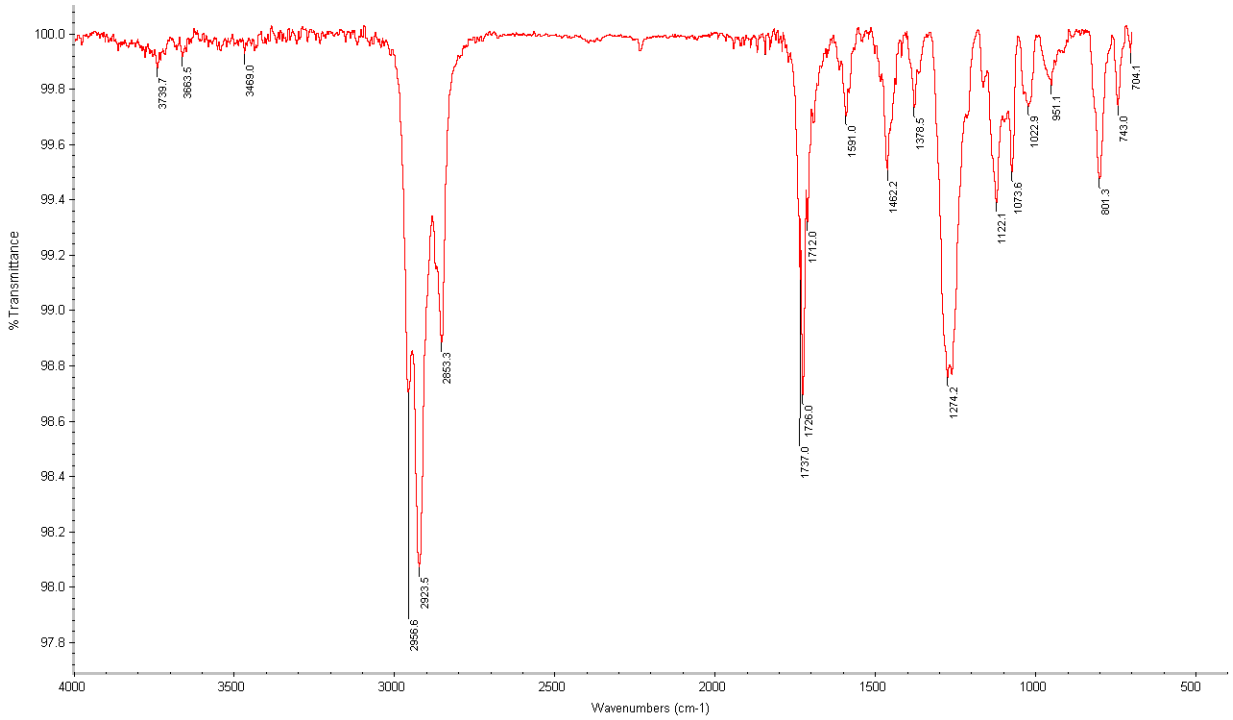

Figure S3. IR spectrum of $F_{48}H_7$(COOH)PcZn, **3**.

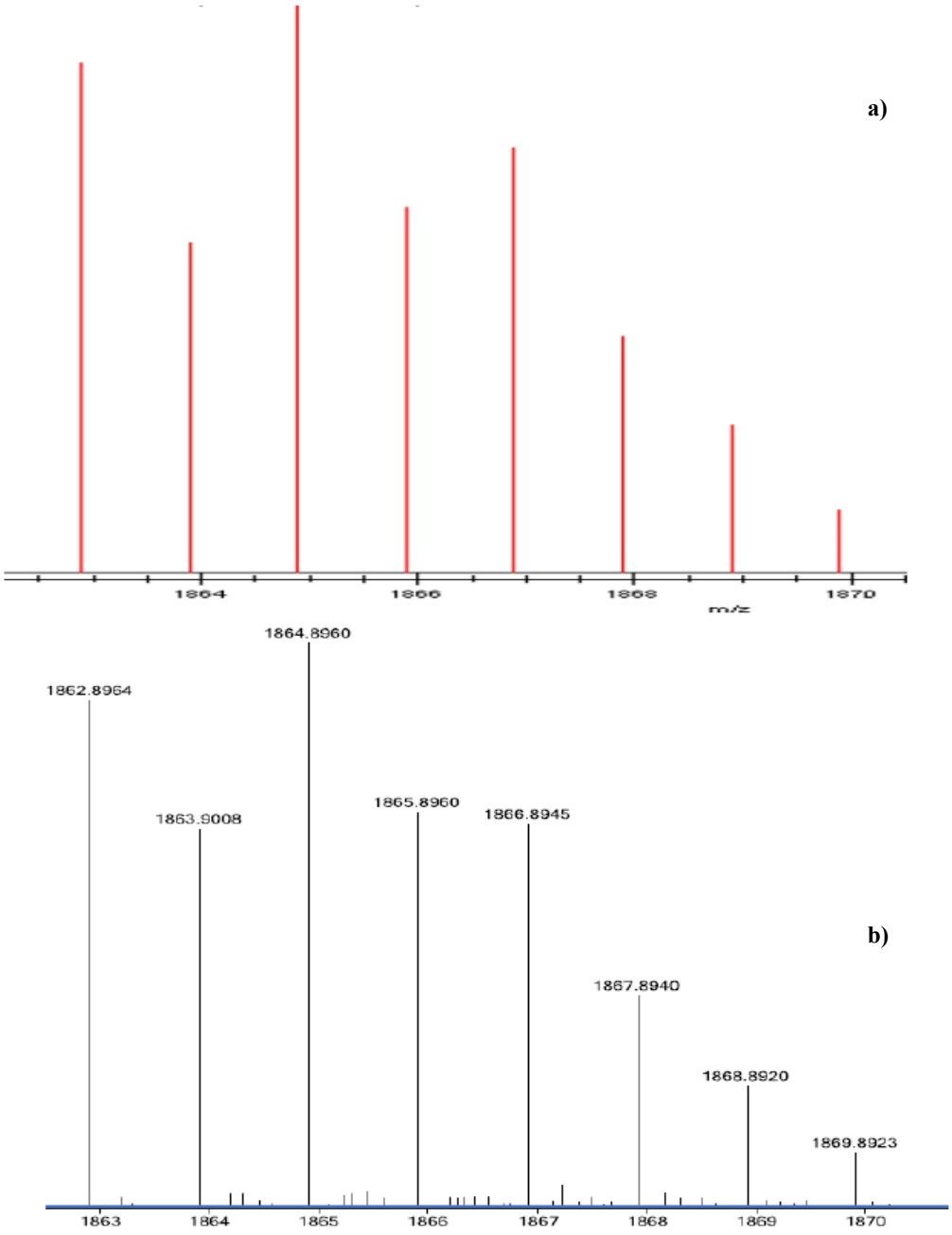

Figure S4. High Resolution Mass Spectrum of **3**. Calculated **a)** and observed **b)** isotopic patterns for **3**•Cl^-, $[F_{48}H_7(COOH)PcZnCl]^-$ Molecular weights: calculated: 1862.8938; observed: 1862.8964.

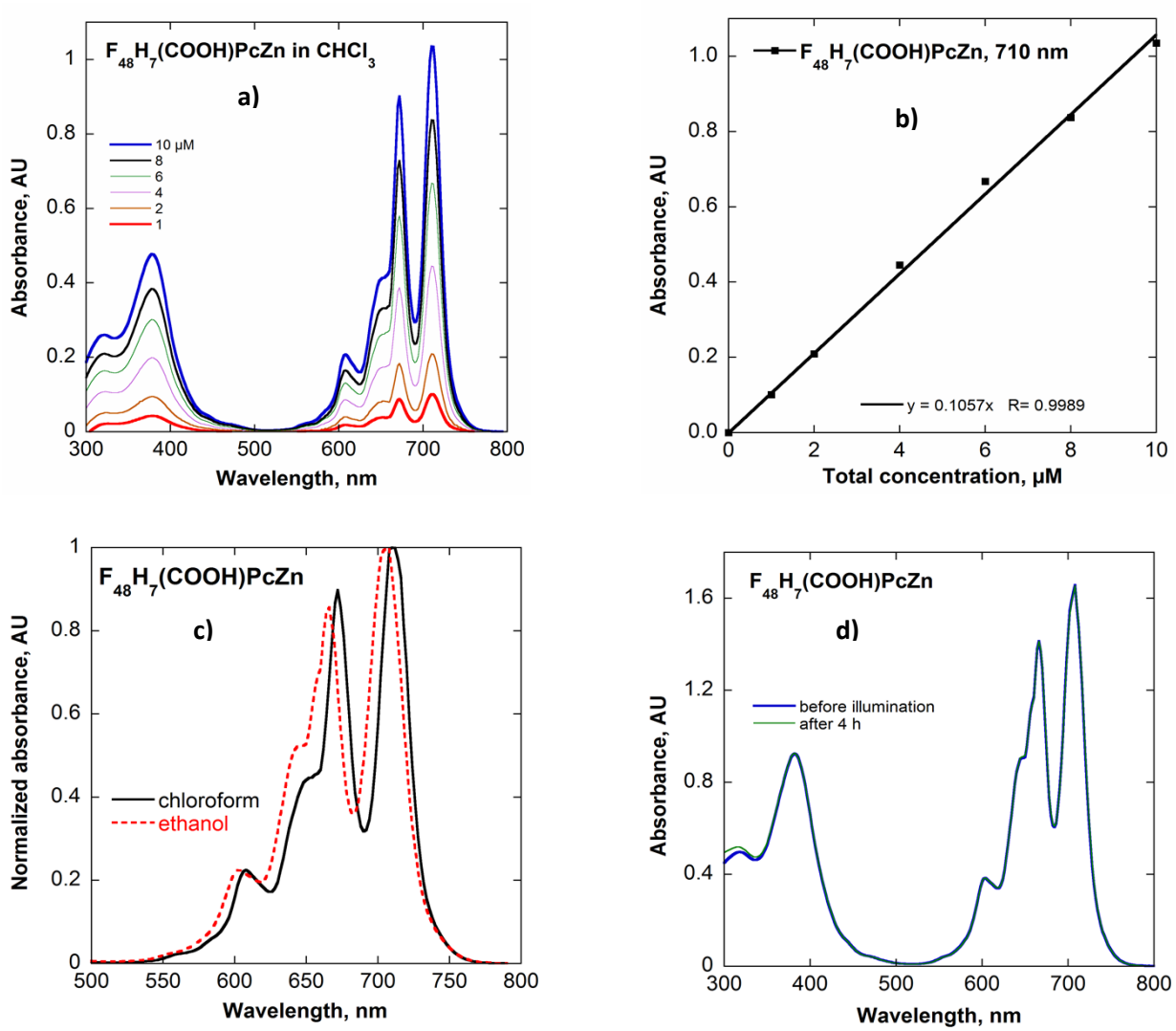

Figure S5. UV-Vis electronic absorption spectra of $F_{48}H_7$(COOH)PcZn, **3**. a) **3** (1-10 μM) in chloroform; b) Lambert-Beer plot of the Q-band absorption maxima; c) normalized absorption spectra of **3** in chloroform and ethanol; d) Time-dependent UV-Vis of **3** during the photooxidation of *β*-citronellol in ethanol up to the time of complete consumption of O_2, ~14,400 sec. (4 hrs.). No catalyst decomposition is observed. The Q-band absorptions, shifted toward the red region of the spectrum are consistent with the expected effect of peripheral substitution by electron-widthdrawing groups. See, for example, N. Kobayashi, H. Ogata, N. Nonaka, E. A. Luk'yanets, *Chemistry*. 2003, **9**, 5123-5134. The Q-bands split is due to the loss of the typical

phthalocyanine 4-fold symmetry, as noticed previously, for example, for a related zinc-phthalocyanine, conjugated with deoxyribonucleosides. See B. Das, E. Tokunaga, M. Tanaka, T. Sasaki, and N. Shibata, *Eur. J. Org. Chem.* 2010, **2010**, 2878-2884.

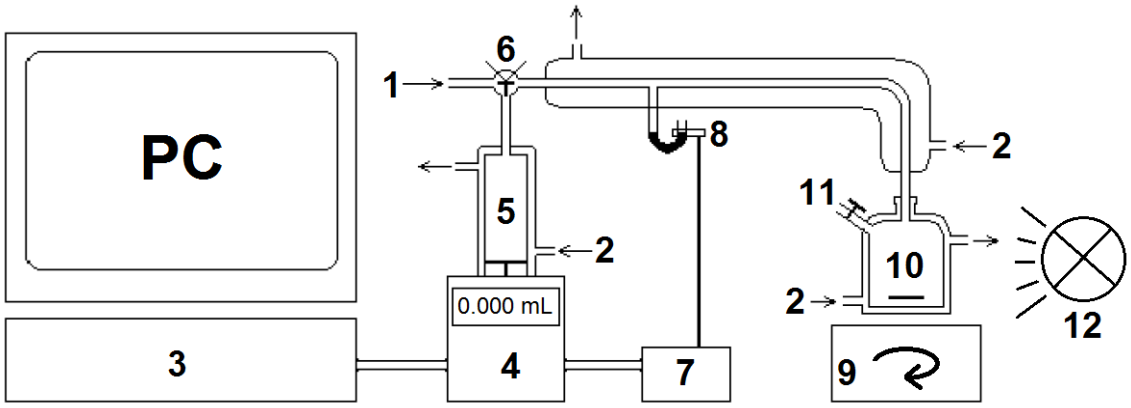

Figure S6. Schematic representation of the catalytic oxidation equipment. (1) Gas inlet; (2) thermostated water inlet; (3) computer-monitored oxygen consumption; (4) Dosimat; (5) oxygen reservoir; (6) three-way tap; (7) electrical couple for Dosimat dispenser control; (8) mercury switch; (9) magnetic stirrer; (10) 100 mL double-walled glass reaction vessel; (11) solution inlet; (12) light source. See also Reference 5.

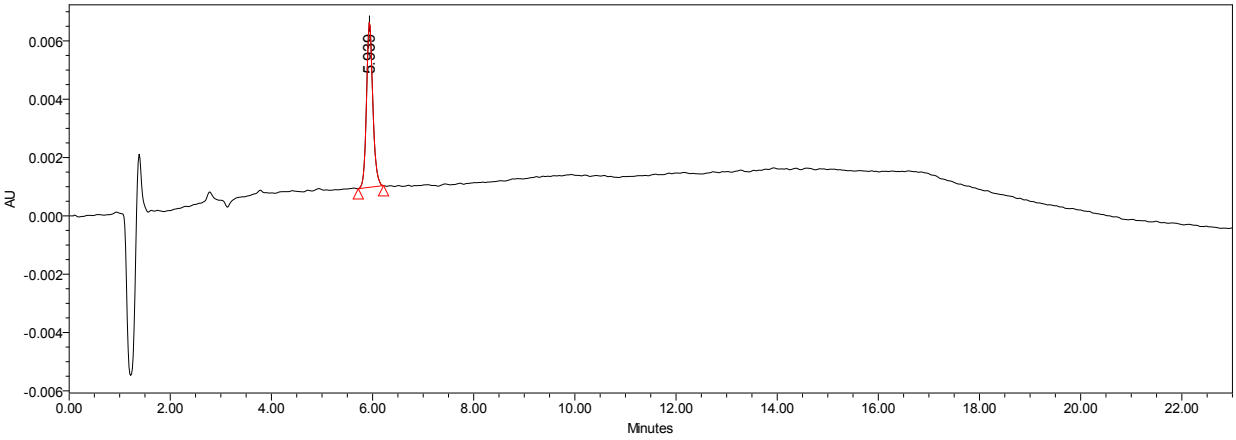

Figure S7. HPLC trace of GRP78 CTO, **9.**

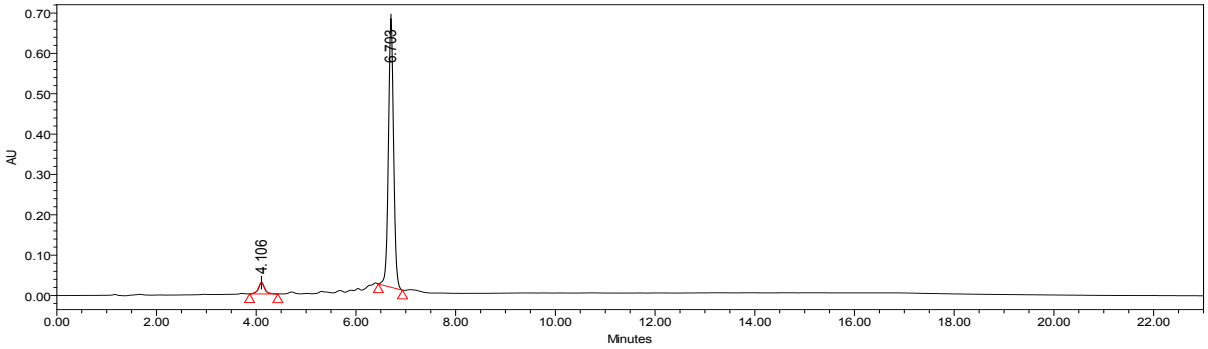

Figure S8. HPLC trace of GRP78 DNA, **7.**

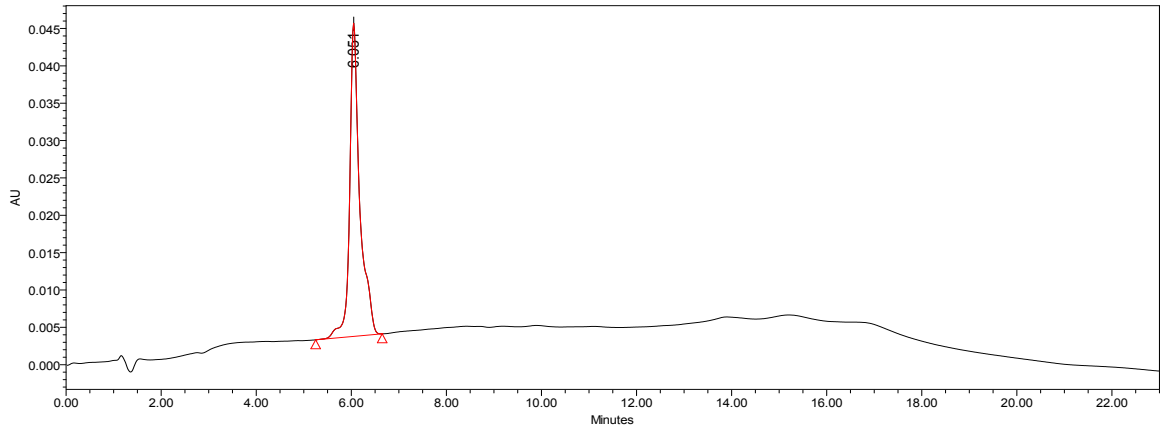

Figure S9. HPLC trace of GRP78 mRNA, **8.**

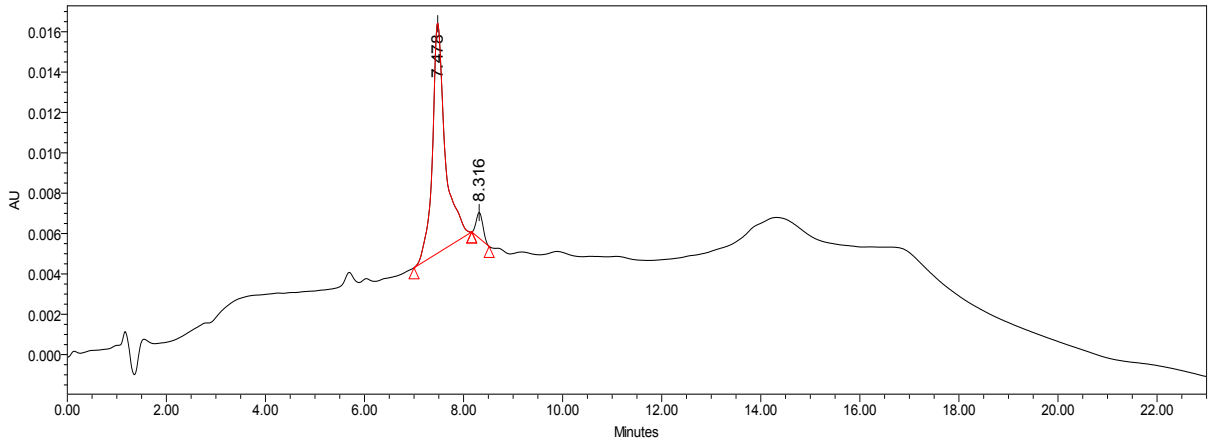

Figure S10. HPLC trace of $F_{48}H_7PcZn$-CTO bioconjugate, **6.**

Table S1. Electron spray mass spectrometry data for oligonucleotides **6-10**.

	Sequence	Calculated Mass	Observed Mass
6	$F_{48}H_7PcZn$ –CONH-C6- 5'-CTTCATCTTGCCGGCGCT-3'	7410.6	7411.4
7	5'-AGCGCCGGCAAGATGAAG-3'	5582.7	5583.1
8	5'-AGCGCCGGCAAGAUGAAG-3'	5856.1	5856.1
9	5'-CTTCATCTTGCCGGCGCT-3'	5417.5	5417.1
10	NH_2-C6-5'-CTTCATCTTGCCGGCGCT-3'	5596.7	5596.8

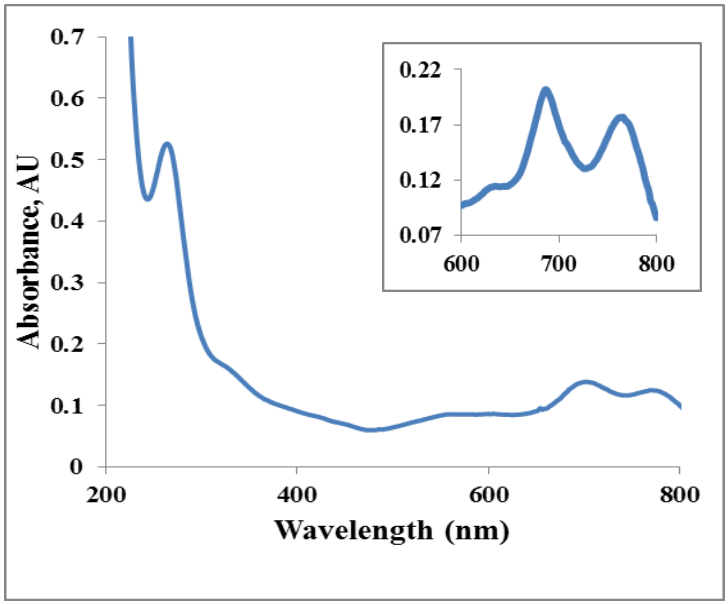

Figure S11. UV-Vis electronic absorption spectra of $F_{48}H_7PcZn$-CTO bioconjugate, **6**

Table S2. Thermal denaturation data of duplexes formed with **6-9**

Molecule	Sequences	T_m (°C)	%H
6	5'-$F_{48}H_7$PcZn-CONH-C6-CTTCATCTTGCCGGCGCT-3'	75	19
7	3'-GAAGTAGAACGGCCGCGA-5'		
6	5'-$F_{48}H_7$PcZn-CONH-C6-CTTCATCTTGCCGGCGCT-3'	72	19
8	3'-GAAGUAGAACGGCCGCGA-5'		
9	5'-CTTCATCTTGCCGGCGCT-3'	73	20
7	3'-GAAGTAGAACGGCCGCGA-5'		
9	5'-CTTCATCTTGCCGGCGCT-3'	74	14
8	3'-GAAGUAGAACGGCCGCGA-5'		

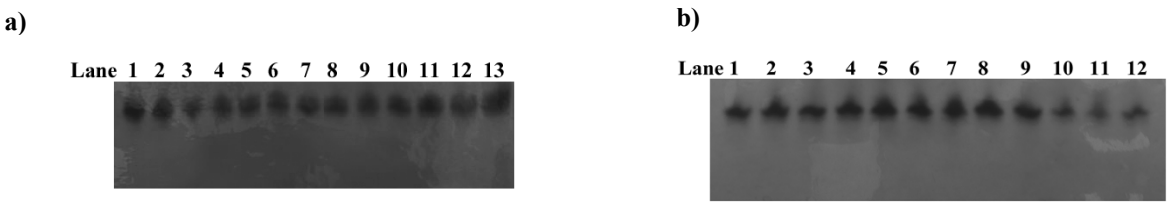

Figure S12. 24% PAGE for the photooxidation and piperidine cleavage reactions. a) **9:7** and b) **9:8**. Lane 1: light, but no O_2. Lane 2: no light, but with O_2. Lanes 3-12 or 13, time points from 0-12 hrs.

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