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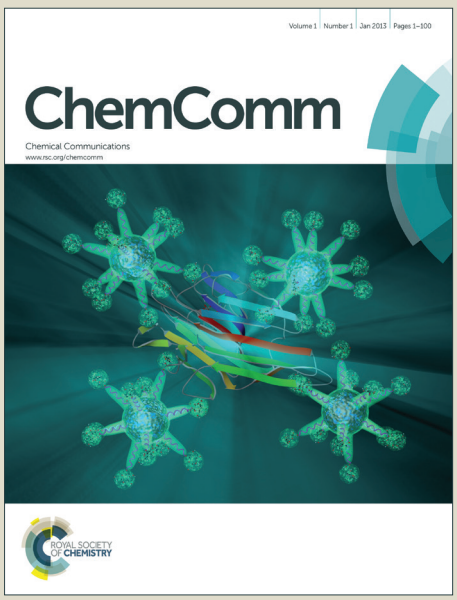

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Chemically Robust Fluoroalkyl Phthalocyanine-Oligonucleotide Bioconjugates and their GRP78 Oncogene Photocleavage Activity

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The first representative of functionalized fluoroalkyl phthalocyanines, $F_{48}H_7$(COOH)PcZn, is reported. The complex generates $^1O_2$ affording long-lasting photooxidation of an external substrate without self-decomposition. The carboxylic group couples with an antisense oligonucleotide targeting GRP78 oncogenes, resulting in the $F_{48}H_7$PcZn-cancer targeting oligonucleotide (CTO). The bioconjugated fluorophthalocyanine effectively hybridizes complementary GRP78 DNA and mRNA sequences. Piperidine cleavage assays reveal desired photochemical oligonucleotide oxidative degradation for both $F_{48}H_7$PcZn-CTO:DNA and $F_{48}H_7$PcZn-CTO:mRNA hybrids. This new materials strategy could be extended to other functional fluorinated phthalocyanines - antisense oligonucleotide combinations for long-lasting oncogene-targeting photodynamic therapy.

Photodynamic therapy (PDT) uses photosensitizers to produce reactive oxygen species, ROS (e.g. singlet oxygen, $^1O_2$), that kill tumor cells.[1] Advantageously, PDT does not trigger immunosuppressive or myelosuppressive effects that accompany surgery, chemotherapy or radiation treatments. Historically, the first successful clinical application of PDT in oncology was demonstrated over 100 years ago,[2] with the first PDT drug approved for bladder cancer in 1993.[3] However, unfavorable photosensitizer properties that lower therapeutic index, such as: poor water solubility, limited extinction coefficients in the maximum tissue penetration NIR region, poor clearance from normal cells and photostability contributed to limited approval of PDT drugs for clinical use. Moreover, poor tumor-targeting results in dispersion in all tissue types, depressing efficiency and enhancing harmful side-effects. Most of the photosensitizers used in oncology are based on the tetrapyrrole scaffold, such as, the porphyrins, chlorins, corroles and phthalocyanines.[1] The phthalocyanines, in particular, are easily modified while exhibiting high thermal and chemical stability, in addition to intense light absorption toward the advantageous red spectral region.[4]

In order to help mitigate current photosensitizers' limitations, we report the first example of a new class of *functionalized* fluoroalkyl phthalocyanine photosensitizers, $F_{48}H_7$(COOH)PcZn, **3**, in which most of the C-H bonds of the Pc macrocycle are replaced by aromatic and aliphatic C-F bonds. The fluorinated phthalocyanines, $F_x(R_F)_y$PcM , (F_x = fluorine, R_F = perfluoro alkyl group, $x + y \leq 16$, Pc = phthalocyanine and M = metal) constitute an important class of chemically robust photosensitizers.[5] The fluorinated alkyl substituents, R_F, in contrast to aromatic F groups are expected to enhance the stability of the Pc macrocycle towards nucleophilic attack as the y/x ratio increases, as well as the resistance to attack by ROS that the Pcs produce during photodynamic events.[6] Thus, partial replacement of aromatic F in perfluorophthalocyanine, F_{16}PcM (M = Zn; x = 16, y = 0), with *iso*-perfluoroalkyl groups (R_F) to give octakis-(perfluoro-*i*-C_3F_7)-(perfluoro)PcM, F_{64}PcM (x = y = 8) was shown to improve the Pc's solubility in organic solvents, depress the HOMO-LUMO gaps, thereby extending the Q-bands absorption in the 600-900 nm NIR region, while retaining high ~10^5 molar extinction coefficients favouring efficient $^1O_2$ production.[7] The R_FPc macrocycle scaffold's resistance to degradation prompted their use as artificial enzymes,[8] anti-cancer drugs,[9] photosensitizers for degradation of azo dyes,[10] and as redox catalysts.[11] However, the lack of the R_FPc's specific tissue distribution suggested the need for a targeting approach in our PDT applications. The enhanced tissue localization of photosensitizers is particularly useful for potentially increasing their therapeutic index. Strategies for specific tissue targeting include anchoring protio Pcs onto bio-probes, following their functionalization with –COOH, -NH_2, -SO_3H, -X (X = Cl, Br, I) groups[12,13] that thus facilitate Pc conjugation with carbohydrates,[14] nucleosides,[15] oligonucleotides,[16] peptides[17] and proteins.[18] However, these approaches offer a limited therapeutic index for effective and long-lasting PDT due to the decomposition of the protio Pcs by the ROS they produce.[19] Moreover, chemically less stable hydrocarbon-based Pcs coupled to short, arbitrary oligonucleotide sequences have been reported in the literature.[19] However, to the best of our knowledge, coupling a chemically

robust fluoro Pc with an oligonucleotide vector, Figure 1, targeting a DNA or mRNA oncogene, has not been reported.

The cancer-targeting oligonucleotide, CTO, was selected from the antisense DNA sequence exhibiting potent Glucose Regulating Protein 78, GRP78 knockdown in neonatal rat cardiomyocytes.[20] GRP78 functions as a chaperone that regulates protein folding events in the lumen of the endoplasmic reticulum (ER) of all cell types.[21]

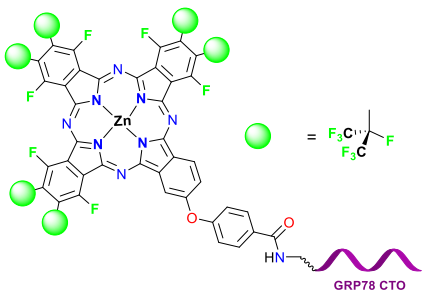
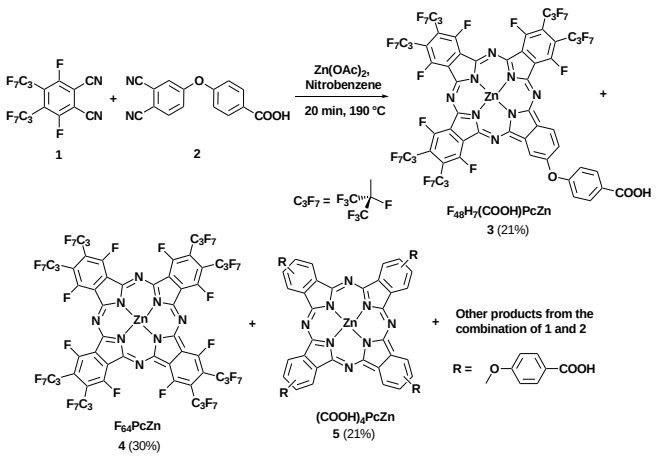


Figure 1. $F_{48}H_7PcZn$-GRP78 cancer-targeting oligonucleotide (CTO) bioconjugate, **6**.

In cancer, GRP78 is overexpressed and localized on the cell surface where it exhibits a myriad of signaling activities related to cancer initiation, production and metastatic spread.[22] The GRP78 oncogene is thus an excellent target for the development of potent and selective anti-cancer strategies.[23]



Scheme 1. Microwave-assisted synthesis of $F_{48}H_7(COOH)PcZn$, **3**.

The $F_{48}H_7(COOH)PcZn$, **3**, was prepared in 21% isolated yield by reacting perfluoro phthalonitrile **1**,[24] with a carboxy phthalonitrile **2**,[25] in a microwave reactor, Scheme 1. Its composition and purity were confirmed by ^1H, ^{19}F NMR and HRMS (see Supporting Information).

Importantly, **3** generates $^1O_2$ as evidenced by the aerobic photo-hydroperoxidation of β-citronellol (Figures 2a,b). The catalyst nuclearity and stability under reaction conditions were established via time-dependent UV-Vis spectroscopy. The solvent-independent Q-bands intensities and linearity of the Lambert-Beer plot for **3** (Figure S5) reveal its lack of aggregation, a key factor for avoiding the shortening of the Pc excited states lifetimes via intermolecular interactions which lowers $^1O_2$ production efficiency. Importantly, **3** remains unchanged (>99%) at the end of the photooxidation reaction thus establishing its reactivity without self-decomposition. Taken together, this data demonstrates the ability of the functionalized fluorophthalocyanine to exhibit single-site structural characteristics in solution and to photooxidize an external substrate without being decomposed by the $^1O_2$ it produces.

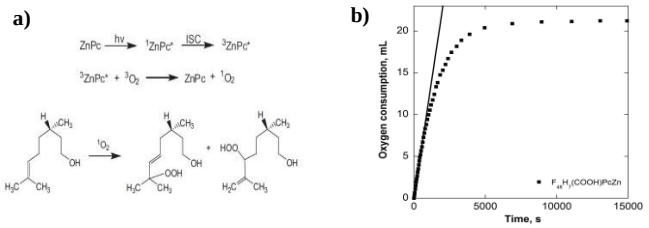


Figure 2. a) Photooxidation of β-citronellol in EtOH by **3,** ("ZnPc"). ISC = intersystem crossing; b) Time-dependent O_2 titration at 25 ºC. No reaction occurs in the absence of either light or O_2. See also Figure S6. The O_2 was consumed in the calculated stoichiometric amount.

The reaction obeys pseudo-first order kinetics with an initial reaction rate of 27.8 μmol O_2 min^{-1} that corresponds to a turnover frequency 460 mmol citronellol s^{-1} mol Pc^{-1}. This rate is consistent with the 33.6 μmol O_2 min^{-1} value reported for the homoleptic $F_{64}PcZn$, **4**.[6,26]

In contrast, the hydrocarbon-based Pc-conjugates are not stable, as exemplified by the loss of Q-bands intensities in the case of a protio PcCo-oligonucleotide conjugate that is exposed to ROS.[19a] The Pc complexes with Zn and Al are presumably active in generating singlet oxygen, but the stability of the Pc is not reported.[19b]

The carboxy group of the Pc catalyst, **3**, was next coupled with the amino group of the solid-supported CTO, 3'-TCGCGGCCGTTCTACTC-5'-C_6-NH_2, **10**, using conventional amide coupling conditions[27] to yield after HPLC purification the bioconjugate $F_{48}H_7PcZn$-CTO, **6**, in isolated purity > 95% (see Supporting Information for synthesis and characterization). The hybridization of bioconjugate **6** with complementary GRP78 DNA, **7** and mRNA, **8** sense strands, performed next, afforded the duplexes $F_{48}H_7PcZn$-CTO:DNA, **6:7**, and $F_{48}H_7PcZn$-CTO:mRNA, **6:8**. These hybrids, soluble in phosphate buffers, exhibited stable (T_m = 75 and 72 °C, respectively) temperature-dependent duplex to single-strand transitions (Table S2). Taken together, these results unambiguously establish the ability of $F_{48}H_7PcZn$-CTO to target and bind to complementary GRP78 DNA and mRNA, affording thermally stable duplex structures, similar to the ones formed by the native controls (Table S2).

Given the ability of $F_{48}H_7(COOH)PcZn$, **3**, to generate and survive $^1O_2$ production, the photoreactivity of $F_{48}H_7PcZn$-CTO, **6**, hybridized with complementary GRP78 DNA and mRNA, i.e. **6:7** and **6:8**, respectively, was explored. This reactivity is critically important for establishing the ability of bioconjugates to generate singlet oxygen and the capacity of the latter to

oxidatively cleave the thermally stable oligonucleotides. Thus, illumination with a 300 W halogen light for 12 hrs in the presence of O_2 followed by hot piperidine treatment induced site-specific cleavage along the oligonucleotide backbone at oxidized sites,[28] Figure 3. In contrast, no degradation occurred in the absence of light and/or O_2, or using unlabeled controls (see also Figure S12).

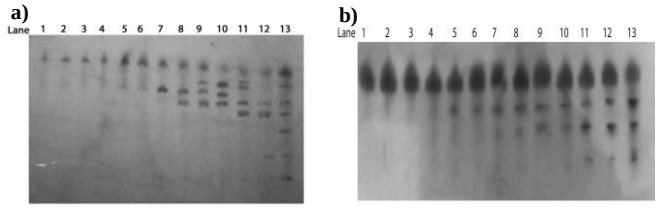


Figure 3. 24% PAGE for the photooxidation and piperidine cleavage pattern of a) **6:7** and b) **6:8**. Lane 1: light, but no O_2. Lane 2: no light, but with O_2. Lanes 3-13, time points from 0-12 hrs.

Conclusions

In conclusion, an entry point into a new family of highly fluorinated phthalocyanines was established. A highly fluorinated, functionalized molecule was synthesized and shown to generate $^1O_2$ without self-decomposition, underscoring its potential in PDT[1] and other applications.[29] Its coupling with a cancer-targeting oligonucleotide vector proved useful in directing GRP78 DNA and mRNA oncogene stable binding, but also cleavage upon aerobic illumination. The photoactive and chemically robust fluorophthalocyanine-CTO bioconjugates encompass a new class of potential anticancer agents. Variations in the fluorinated phthalocyanine structure, coupled with variations in the types of vectors may facilitate the targeting of a broad range of oncogenes and cancer types.

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Notes and References

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Electronic Supplementary Information (ESI) available: Experimental methods, synthetic details and characterization data including NMR, IR, UV-Vis and LCMS

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