Unexpected Disparity in Photoinduced Reactions of C60 and C70 in Water with the Generation of O2•− or 1O2

Korinne Liosi, Anton J. Stasyuk, Fabio Masero, Alexander A. Voityuk, Thomas Nauser, Victor Mougel,* Miquel Sola,* and Yoko Yamakoshi*

Cite This: JACS Au 2021, 1, 1601−1611

ABSTRACT: Well-defined fullerene-PEG conjugates, C60-PEG (1) and two C70-PEG (2 and 3 with the addition sites on ab-[6,6] and cc-[6,6]-junctions), were prepared from their corresponding Prato monoadduct precursors. The resulting highly water-soluble fullerene-PEG conjugates 1−3 were evaluated for their DNA-cleaving activities and reactive oxygen species (ROS) generation under visible light irradiation. Unexpectedly, photoinduced cleavage of DNA by C60-PEG 1 was much higher than that by C70-PEG 2 and 3 with higher absorption intensity, especially in the presence of an electron donor (NADH). The preference of photoinduced ROS generation from fullerene-PEG conjugates 1−3 via the type II (energy transfer) or the type I (electron transfer) photoreaction was found to be dependent on the fullerene core (between C60 and C70) and functionalization pattern of C70 (between 2 and 3). This was clearly supported by the electron transfer rate obtained from cyclic voltammetry data and computationally estimated relative rate of each step of the type II and the type I reactions, with the finding that type II energy transfer reactions occurred in the inverted Marcus regime while type I electron transfer reactions proceeded in the normal Marcus regime. This finding on the disparity in the pathways of photoinduced reactions (type I versus type II) provides insights into the behavior of photosensitizers in water and the design of photodynamic therapy drugs.

KEYWORDS: water-soluble fullerene, reactive oxygen species, photoinduced electron transfer, photoinduced energy transfer, photosensitizer, photodynamic therapy

INTRODUCTION

Applications of fullerenes (C60 and C70) as photosensitizers (PSs) in photodynamic therapy (PDT) have been considered for decades.1−3 Fullerenes offer suitable photophysical properties as PDT-PSs: (1) they generate reactive oxygen species (ROS) in high quantum yields (e.g., quantitative singlet oxygen (1O2) in organic solvents)4−6 and (2) they can be excited by light with low-energy in the vis-NIR region, which can penetrate deeper into tissues, while being less harmful. In addition, there is no significant toxicity reported for C60 and C70 in the absence of light. Among fullerenes, C70 has been regarded as an excellent PDT-PS since it shows relatively higher absorption intensity than C60 in the visible region. Furthermore, its triplet excited state has a longer lifetime (τ > 2.2 ms in benzene and 51 ms in toluene) in comparison to C60 (133 μs and 0.41 ms, respectively).7,8 Indeed, C70 acts as a good photocatalyst for generating 1O2 that affects oxidations under visible light irradiation in CHCl3.9

The understanding of the mechanism for the generation of ROS is key to rationally improving PSs for PDT applications. The generation of ROS from fullerenes was found to proceed mainly via two pathways (type II energy transfer and type I electron transfer, Scheme 1). The type II energy transfer pathway was reported by Foote and co-workers for both C60 and C70 in organic solvents and observed as the quantitative generation of 1O2.4−6 Almost simultaneously, type I electron transfer was reported by Kursic et al. and Foote et al.,10,11 based on the calculated redox potential of the triplet excited state of C60 (3C60•*), which was higher than that in its singlet state.
ground state ($^1$C$_{60}$; $E_1 = −0.42$ V vs SCE in PhCN; $^3$C$_{60}$*: $E_2 = +1.14$ V vs SCE in PhCN). Via this type $I$ pathway, the generation of O$_2$•− and *OH was observed in aqueous solution of C$_{60}$ complexed with poly(vinylpyrrolidone) (C$_{60}$/PVP) using electron spin resonance (ESR) spin-trapping methods, while no O$_2$ generation was observed.12,13 However, the detailed mechanism of the photoinduced ROS generation from fullerences in water remains unclear.14 Such mechanism can vary depending on the structures of water-soluble fullerene materials prepared by complexation and derivatization.2,15,16 In particular, ROS generation from photoexcited C$_{70}$ in aqueous media has not been well-described yet.

Beyond the recent report by the Hamblin group that cationic C$_{70}$ derivatives can be used as PDT-PS that showed photocytotoxicity to cancer cell lines17 and photodynamic antimicrobial activity,18 C$_{70}$ derivatives have not been extensively investigated for PDT applications. This paucity of studies results from the complex generation of multiple C$_{70}$ isomers upon derivatization. Even simple monoderivatization methods such as Diels−Alder cycloadditions19 or 1,3-dipolar cycloaddition reactions (Prato reaction)20 applied to C$_{60}$ produced several isomers (ab-[6,6], cc-[6,6], and dc-[6,6] or dd-[5,6]), while C$_{60}$ usually generates just one [6,6]-isomer due to the higher double bond character of the [6,6]-bond and $I_h$ symmetry of C$_{60}$.21 Such changes in the addition patterns in fullerenes often induce differences in their UV−vis absorption spectra affecting their photophysical properties, especially in ROS generation. Indeed, there were several previous reports on the differences in photophysical properties of C$_{70}$ isomers.22,23 The investigation of the photophysical properties of isolated isomers of C$_{70}$ derivatives is important for advancing their PDT applications.

In this study, we used well-defined, water-soluble C$_{60}$-PEG 1 and C$_{70}$-PEG derivatives 2 and 320 and tested them for photoinduced DNA damage. The generation of ROS (O$_2$ and O$_2$•−) in conjugates 1−3 was measured by ESR in the presence of spin-trapping agents. These results revealed unexpected disparities in both DNA cleavage and the ROS generation (O$_2$, versus O$_2$•−) between these conjugates 1−3. To understand these differences, photophysical (laser flash photolysis) and electrochemical (cyclic voltammetry) measurements were performed. Finally, computational studies were employed to investigate the reaction rate of each step in type $II$ and type $I$ pathways to understand the mechanisms of ROS generation from C$_{60}$-PEG and C$_{70}$-PEG conjugates 1−3.

# EXPERIMENTAL SECTION

## General

NMR spectra were recorded at room temperature on Bruker 400 spectrometer and Bruker 600 spectrometer equipped with a CryoProbe (Bruker BioSpin GmbH). HRMS spectra were recorded on a Bruker MALDI-TOF, Bruker maXis ESI-QTOF spectrometer, and Bruker solarIX spectrometer (Bruker Daltonics GmbH). FT-IR spectra were recorded on PerkinElmer Spectrum One FT-IR spectrometer with a universal ATR sampling accessory (PerkinElmer Inc.). HPLC analyses were carried out using JASCO PU-2080 Plus HPLC pump, JASCO MD2018 Plus detector, and ChromNAV chromatography data system (JASCO, Co.). UV−vis spectra were recorded on a Varian Cary 500 Scan UV−vis NIR Spectrophotometer (Varian Inc.). Column chromatography and analytical TLC were performed on SilicaFlash F60 (230−400 mesh) (Silicycle Inc.) and silica gel 60 F254 TLC (Merck KGaA), respectively. All the solvents used were HPLC grade and purchased from Acros Organic (Thermo Fischer Scientific, Inc.). All used water were Milli-Q water.

### Synthesis of Water-Soluble C$_{60}$-PEG 1 and C$_{70}$-PEG 2 and 3 Conjugates

The bis-carboxylic acid ester precursors for C$_{60}$ and C$_{70}$ were prepared by reported methods.20,29 Obtained [6,6]-adduct of C$_{60}$ (4) and ab-[6,6]- and cc-[6,6]-adducts of C$_{70}$ (5, 6) were converted to C$_{60}$-PEG 1 and C$_{70}$-PEG 2, 3 by deprotection and subsequent conjugation with amino-PEG$_{15}$-Bu ester (Scheme S1). 2: $^3$H NMR (600 MHz, in CDCl$_3$) $\delta$ 1.44 (s, (CH$_3$)$_3$O), 1.49 (s, J = 6.6 Hz, CH$_3$−CH$_2$−CO$_2$−Bu), 4.62−3.65 (m, (CH$_2$−CH$_2$−O)$_3$, 298 Hz). $^{13}$C NMR (150 MHz, in CDCl$_3$) $\delta$ 28.1 (C(CH$_3$)$_3$), 36.3 (CH$_2$−CH$_2$−CO$_2$−Bu), 39.2 (CH−CH$_2$−CO−NH), 39.9 (CH$_3$−CH$_2$−CO−NH), 54.6 ([CH$_2$−CH$_2$−O]), 59.1 (C(CH$_3$)), 61.3 (C(CH$_3$−N)), 63.0 (C−CH$_2$−N), 63.2 (C−CH$_3$−N), 66.9 (O−CH$_2$−CO$_2$−Bu), 70.5 (CH$_3$−CH$_3$−O), 80.5 (C(CH$_3$)), 131.2, 131.3, 131.6, 133.7, 133.8, 137.4, 140.5, 140.6, 142.8, 143.2, 143.3, 143.5, 145.8, 146.3, 146.7, 146.9, 147.0, 147.1, 147.4, 148.8, 149.1, 149.4, 149.5, 149.7, 149.9, 150.2, 150.7, 150.8, 151.0, 151.2, 151.4, 151.8, 155.6 158.1, 170.9; HRMS (MALDI/ESI), matrix: CCA + NA-PFHNA) m/z calculated for [C$_{235}$H$_{325}$NaN$_3$O$_{78}$]+: 4460.1449, found 4460.1479 ([M + Na]+).

### DLS Measurement

Dynamic light scattering (DLS) measurements were performed with an aqueous solution (1.0 mM) of each 1, 2, or 3 at room temperature on a Malvern Nano-Zeta Sizer (Malvern Instruments Ltd.).

### STEM Analyses

Transmission electron microscopy (TEM) images were acquired on a Talos F200X (Thermo Fischer Scientific) instrument operated at 200 kV of accelerating potential in scanning TEM (STEM) mode.

### DNA Cleavage Assay

Supercritical plasmid pBR322 DNA (New England BioLabs) was used as a substrate. To a solution of pBR322 (12.5 μM), 10 μL in 10 mM Tris-HCl buffer (pH 8.0, with 1 mM EDTA), the aqueous solutions of 1, 2, or 3 (final conc.: 0.75 mM) and NADH (final conc.: 10 mM) were added (total volume: 20 μL) and subjected to photoradiation by a green LED (Lumitron ConexPlay, male grin, 5 V, 528 nm, 90 lm-W$^{-1}$) with a distance of 1 cm for 6 h. Subsequently, an aliquot of gycerol solution of bromophenol blue (0.1%, 5 μL) was added subjected to agarose gel electrophoresis (1% agarose in Tris-borate-EDTA (TBE) buffer) in TBE buffer at 100 V for 60 min. The gel was stained with ethidium bromide and visualized by using a transilluminator (Vilber Lourmat Ste) and analyzed by ImageJ.

### Detection of ROS Generations by ESR with Spin-Tracking Reagents

ESR spectra were recorded on a Bruker spectrometer (Bruker BioSpin, GmbH) equipped with microwave bridge X-band ESR. Diethylentriaminepentaacetic acid (DETPAC), NADH, 2,2,6,6-tetramethyl-piperidine (4-oxo-TEMP), and L-histidine were purchased from Sigma-Aldrich Co. Dimethyl sulfoxide (DMSO) was purchased from VWR Internationals, and S-(diethyloxyphosphoryl)S-methyl-l-prolyl-nitroxide (DEPMPO) was purchased from Enzo Life Sciences Inc. For the measurements of O$_2$ generation, C$_{60}$-PEG 1−3 (40 μM) and 4-oxo-TEMP (80 μM) were mixed in 60 mM phosphate buffer (pH 7.0). For the measurements of O$_2$•− generation, C$_{60}$-PEG 1−3 (40 μM), DEPMPO (113 mM), NADH (10 mM), and
DETAPAC (1 mM) were mixed in 60 mM phosphate buffer (pH 7.0) with 20% (v/v) DMSO in the presence or absence of l-histidine (10 mM). Each mixture above was irradiated with a green LED (Lumitonix, PowerBar V3, 528 nm, 93 lm W−1, with a total of 160 lamps assembled in a cylindrical manner with a distance of 4 cm, details are in the Supporting Information).

ESR measurements were carried out in a glass capillary (50 μL micropipet, Blaubrand), which was placed inside a thin-wall precision quartz ESR tube (Wilma, Φ 4 mm × 250 mm). Double integration of ESR spectra was performed with the WINEPR Processing program (Bruker). Measurement conditions: temperature 296 K, microwave frequency 9.78 GHz, microwave power 10 mW, receiver gain 5.0 × 10^4, modulation amplitude 1.00 G, modulation frequency 100 kHz, and sweep time 83.89 s.

Laser Flash Photolysis (LFP)
LFP experiments were carried out on an Applied Photophysics LKS 50 instrument. The solutions were transferred into quartz cuvettes that were sealed by a Young-type valve. Deaeration was carried out by repeated evacuation and flushing with argon. As an excitation laser, the second (332 nm) or the third harmonic (355 nm) of a Nd:YAG-Laser (Quantel Brilliant B) was used. C_{60}-PEG 1 was excited at 532 nm with energies of 20–50 mJ/pulse, C_{70}-PEG 2, 3 were excited at 355 nm with energies of 40–90 mJ/pulse. Ten experiments were averaged per kinetics trace since the measured absorbance differences were very low for C_{60}-PEG 2 and 3.

Cyclic Voltammetry
All measurements were performed under strictly anaerobic conditions in a conventional three electrode single-compartment cell with an SP300 Bio-Logic potentiotstat (Bio-Logic Science Instruments SAS). A Ag/AgCl (Ag/AgCl glue in 0.1 M TBAPF 6/MeCN) reference electrode was used for the experimental setup. The reference electrode was placed in a compartment separated by a Vycor frit. All potentials were referred to internal Fc/Fc+ standard added in the last step of the experiments. Three mm diameter glassy carbon electrodes (GCE, BASi) were used as working electrodes. Electrodes were polished on a wet polishing cloth using a 1 μm diamond suspension before each use. The scan rate was 100 mV s^{−1} unless otherwise noted. The half-wave potentials (E_{1/2}) were defined as the weighted average of the corresponding E_{a} (the potential at which highest current was achieved for a particular cathodic event) and E_{c} (the potential at which highest current for corresponding anodic process was achieved). Unless otherwise noted, 1 mM solutions in toluene–MeCN (10:4:1) containing 0.1 M TBAPF 6 as supporting electrolyte were used. For all CV data, the second of three successive scans was presented.

Computational Details
Geometry optimizations were performed employing the range-separated functional from Handy and co-workers’ CAM-B3LYP and Ahlrichs and Weigend’s Def2-SVP basis set. The empirical dispersion D3 correction with Becke–Johnson damping was employed. Vertical excitation energies were calculated with the same functional and basis set using the Tamm–Dancoff approximation (TDA). This method was proven to give accurate results for charge transfer processes involving fullerenes. Solvent effects were estimated using a COSMO-like polarizable continuum model in monopole approximation considering water as solvent. All calculations were performed with the Gaussian 16 (rev. A03) suite of programs. Full computational details including how species 1–3 and NADH were modeled can be found in the Supporting Information.

RESULTS AND DISCUSSION
Preparation of Water-Soluble C_{60}-PEG and C_{70}-PEG Conjugates
The water-soluble C_{60}-PEG 1 and C_{70}-PEG 2, 3 (Figure 1a) were prepared by the conjugation of amino-PEG_{36}·Bu ester with the C_{60} and C_{70} bis-carboxylic acid precursors (4–6) using hexafluorophosphate benzotriazole tetramethyl uronium (HBTU) as a coupling reagent (Scheme S1). The C_{60}- and C_{70}-PEG (1–3) were purified by reversed phase HPLC and were thoroughly soluble in water (>5 mM). The UV–vis-NIR spectra of C_{2r}-PEG (1–3) in water (Figure 1b) shows that all 1–3 had the absorption in the range of the phototherapeutic window (650–860 nm), with a much higher intensity for C_{70}-PEG 2, 3 in comparison to C_{60}-PEG 1. Computational results reproduce this experimentally observed trend (Figure S14). Compared to the spectra of pristine C_{60} and C_{70} and precursors 4–6 measured in CHCl_{3}, aqueous solutions of 1–3 revealed relatively red-shifted spectra (Figure S13). Similar to the observed situation in precursors 5 and 6, different absorption patterns were found between two isomeric C_{60}-PEG 2 and 3 in the visible region. The observed longest absorption bands for 1–3 were respectively at ca. 720, 680, and 715 nm (inset of Figure 1b). The absorption intensities of 1–3 at 528 nm (maximum wavelength of green LED light, used in a later section) were found to have molar absorption coefficients (ε) of 2570, 14 600, and 10 200 M^{−1}·cm^{−1}, in line with the theoretical calculation results with an absorbance ca. 1.5 times higher for our computed model of 2 than for our computed model of 3 (Figure S14).
Figure 2. (a–c) DLS in volume distribution of C₆₀-PEG 1 (a), C₇₀-PEG 2 (b), and C₇₀-PEG 3 (c) in aqueous solution (1.0 mM). Mean (width) [nm]: (a) 9.57 (3.0), (b) 8.87 (3.4), and (c) 10.67 (2.8). (d–f) STEM images of C₆₀-PEG 1 (d), C₇₀-PEG 2 (e), and C₇₀-PEG 3 (f) in aqueous solution (0.2 mM).

Morphology Analyses by DLS and STEM

The combination of hydrophilic functionalizing groups and the hydrophobic fullerene surface may result in amphiphilic properties of compounds 1–3. These compounds are hence expected to form micelle-type aggregates in water, which were confirmed in the previous study on compound 1 by surface tension measurements. To study their assembly pattern, DLS measurements and STEM imaging were employed. The DLS results in water showed that the conjugates 1–3 were dispersed well in water by forming aggregates with mean diameters of ca. 9–11 nm with a single mode distribution (Figure 2a–c). It is known that the estimated length of the PEG₃₆ chain used in this study was, in a linear form, approximately 13.4 nm in the all-anti conformation and 9.2 nm in the all-gauche conformation. The hydrodynamic diameters of particles of compounds 1–3 (ca. 10 nm mean) observed by DLS suggested that the PEG moieties in the micelle-type aggregations of 1–3 are tangled (Figure S15).

The STEM images of 1–3 (ca. 0.2 mM in water) were measured to obtain the morphological information on 1–3 in water. As shown in Figure 2d–f (and Figures S16–S18), all samples showed spherical-type aggregates, supporting their micelle-type assembly. While C₆₀-PEG 1 formed relatively more uniform and round morphologies, C₇₀-PEG 2 and 3 appeared to be rather random. Diameter analyses of the aggregates of the compounds 1–3 provided the mean in the range of 13–17 nm, in a good agreement to the DLS results (Figure S19).

Photoinduced DNA Cleavage

To preliminarily evaluate the efficiency of C₆₀-PEG and C₇₀-PEG 1–3 as PSs for PDT, photoinduced DNA cleavage tests were performed using a standard DNA (supercoiled pBR322) to quantify the formed nicked DNA (form II) in comparison to the intact ones (form I). A green diode with intensity maximum at 528 nm was used as a light source and photolysis was carried out for 6 h in the absence or presence of an electron donor (NADH). Results are shown in Figure 3. Sufficient DNA cleavage by 1–3 was observed (lanes 3–5) in comparison to the control experiment (lane 1). The amount of nicked form II produced by compounds 1–3 were 28%, 38%, and 25%, respectively. Considering the relative absorption intensity of 1–3 at 528 nm (ca. 1:5.7:4.0), the quantum yield in photoinduced DNA damage by C₆₀-PEG 1 was higher, while the damages by C₇₀-PEG 2 and 3 were similar to each other.

DNA damage was significantly enhanced in the presence of NADH (Figure 3a, lanes 6 (87%), 7 (50%), and 8 (57%); Figure 3b) in all C₇₀-PEG conjugates, presumably due to the stronger reactive species generated via the electron transfer pathway. As described in Figure 3c, there are two possible pathways for DNA damage involving type I electron transfer reaction; (1) the generation of radical species of substrate (DNA), or (2) the generation of highly reactive •OH via O₂⁻⁻. Interestingly, the DNA cleavage by C₆₀-PEG 1 was much more enhanced (3.1 times) by the addition of NADH, resulting in a stronger DNA cleavage than 2, which were also enhanced 1.3 and 2.3 times (Figure 3b). The results indicated...
that in the cases of compounds 1 and 3, electron-transfer photoreaction proceeded efficiently via either of these pathways, in contrast to compound 2.

It is known that \( ^1\text{O}_2 \) is a key ROS in photoinduced DNA cleavage by type II pathway,\(^{19,20}\) while the electron transfer to DNA or generation of \( ^1\text{O}_2^- \) as a precursor of highly reactive •OH are the keys in the DNA cleavage by type I pathway (Figure 3c).\(^{38–42}\) To investigate the current phenomena more clearly, the generations of \( ^1\text{O}_2 \) and \( ^1\text{O}_2^- \) from photoexcited compounds 1–3 were estimated by ESR spin-trapping methods. Our aim is not to investigate the mechanism of DNA cleavage but to observe different types of ROS generation from the three fullerene-PEG derivatives.

**ESR Measurement of Photoinduced ROS (\( ^1\text{O}_2 \) and \( ^1\text{O}_2^- \)) Generation**

The photoinduced generation of \( ^1\text{O}_2 \) and \( ^1\text{O}_2^- \) from photoexcited C\(_{2n}\)-PEGs 1–3 were measured in aqueous media by ESR using corresponding spin-trapping agents. The \( ^1\text{O}_2 \), which can be generated via a type II energy transfer reaction (Scheme 1), was measured using 4-oxo-TEMP (Figure 4b). Upon irradiation by green LED (528 nm max), the peaks corresponding to 4-oxo-TEMPO were observed in the solution of 1–3 in a time-dependent manner (Figures 4a and S22–S25). The relative amount of \( ^1\text{O}_2 \) was estimated by double-integration analyses of ESR signals in Figure 4a to show that the generation of \( ^1\text{O}_2 \) from C\(_{70}\)-PEG 2 was significantly higher than from C\(_{60}\)-PEG 1 and C\(_{70}\)-PEG 3 (Figure 4b). This result was in good agreement to the results of the photo DNA cleavage assay in the absence of NADH, where stronger DNA damage was observed with 2 in comparison to 1 and 3 (lanes 3–5 in Figure 3a). These results suggested that there was a strong correlation between photo DNA cleavage by 1–3 (in the absence of electron donor) and \( ^1\text{O}_2 \) generation produced via a type II energy transfer mechanism.

Next, the photoinduced \( ^1\text{O}_2^- \) generation was measured in aqueous solutions of 1–3 in the presence of an electron donor (NADH) by ESR using DEPMPO as a spin-trapping reagent (Figure 5). As shown in Figure 5a and b, \( ^1\text{O}_2^- \) generation was observed from all compounds 1–3 in a similar range with slight differences and in a time-dependent manner (Figures S26–S29). As shown in Figure 5c, the observed \( ^1\text{O}_2^- \) was considered to be a sum of the \( ^1\text{O}_2^- \) generated through two pathways: (1) direct generation through type I electron transfer and (2) reduction of \( ^1\text{O}_2 \) first generated via type II energy transfer. To exclusively detect only the \( ^1\text{O}_2^- \) generated via type I electron transfer, \( ^1\text{O}_2^- \) generation was measured in the presence of L-histidine, a \( ^1\text{O}_2 \) quencher. As shown in Figure 5b, the amount of \( ^1\text{O}_2^- \) generated in the presence of L-histidine was significantly decreased in case of C\(_{70}\)-PEG 2, suggesting that the \( ^1\text{O}_2^- \) generation from compound 2 involved the type II energy transfer pathway.

The results clearly indicated that the preferred pathways in the photoinduced reactions of compounds 1–3 were different; the photoreaction of C\(_{70}\)-PEG 2 primarily involved the type II energy transfer mechanism, while C\(_{60}\)-PEG 1 and C\(_{70}\)-PEG 3 preferred the type I electron transfer pathway, providing the different type of ROS generation related to the photoinduced DNA cleavage. To understand the mechanisms of these unexpected differences in these three fullerene derivatives 1–3, experimental investigations were employed: (1) laser flash photolysis to estimate the relative lifetime of the triplet excited states and (2) cyclic voltammetry to obtain the redox potentials and the electron transfer rate of corresponding precursors 4–6. Furthermore, computational calculations were performed to estimate the relative rate for each step in type II and type I reactions.

**Lifetime of the Triplet Excited State**

The photophysical properties of 1–3 were measured by nanosecond laser flash photolysis. The decay of their triplet-excited states (\( ^3\text{C}_{2n}^* \)) in water is shown in Figure 6. C\(_{60}\)-PEG 1 was excited with 532 nm (20–50 mJ) and C\(_{70}\)-PEG 2 and 3 were excited with 355 nm (40–90 mJ), because only minor signals (<1 \times 10^{-3} AU) were detected in C\(_{60}\) isomers with 532 nm excitation even at laser energies > 150 mJ/pulse. In all three cases and for the majority of wavelengths tested, a double exponential decay was observed, with the faster rate constant (\( k_{\text{fast}} \)) > 10^6 s^{-1} and the slower (\( k_{\text{slow}} \)) around 10^5 s^{-1} (Figure 6, Table 1). The observed rate constants were independent of the wavelength. The initial buildup had an apparent rise time of around 10 ns. Given the 5–10 ns width of the laser pulse, the build-up kinetics are too fast to be resolved with our experimental setup.

During the initial absorbance increase, i.e., the buildup of the transient(s) observed, we did not observe substantial fluorescence. It appears that the intersystem crossing (ISC)
has a high quantum yield, in line with reported data. The double-exponential decay observed implies either two consecutive or two independent decay processes. The mechanism cannot consist only of two parallel reactions of the same transient species. Guldi reported that water-soluble fullerenes tend to form clusters and that their triplet-to-singlet ($\mathcal{T}_{C_{2n}} \rightarrow \mathcal{S}_{C_{2n}}$) decay rate was faster than for isolated monomer species. In that original work, surfactants were used to measure the decay rate of monomers, and half-lives of 50–70 $\mu$s were found. We find half-lives ($t_{1/2} = \ln(2)/k$) of around 0.2 $\mu$s for all fast processes. The slower process has half-lives of 2, 7, and 4 $\mu$s for 1, 2, and 3, respectively (Table 1). Within the framework given above, we suspect that the faster process is coupled to the decay of fullerene assembled in micelles, while the slower process is a measure of the decay of fullerene monomers. The latter process was roughly 1 order of magnitude faster than reported by Guldi with similar structures. Additionally, we observed less than 1 order of magnitude lower absorption signals. We do not know if this is related to the fact that Guldi’s monomer species were dissolved with surfactants, which would imply that his experiments were carried out with suspensions, not solutions. As the triplets appear to be more stable in organic solvents (150 and 100 $\mu$s for C$_{60}$ and C$_{70}$), a local lipophilic embedding of the fullerenes by surfactants in the original work might have increased the observed half-lives. More important for our purpose, however, is the longer half-lives of the monomers of 2 and 3 as compared to 1. A longer half-life increases the probability for intermolecular energy transfer, for example by formation of $^{3}\text{O}_2$. Alternatively, direct
excitation of a biomolecule or electron transfer may occur in PDT.

**Redox Potential and Electron Transfer Rate**

In a type I mechanism, the generation of \( \text{O}_2^{**} \) from photoexcited fullerenes is directly related to the redox potential of the \( \text{C}_{2n-6}^{*-}/\text{C}_{2n}^{**} \) couple. As the redox properties of 1–3 mainly arise from the fullerene core, we investigated the electrochemical properties of the analogous derivatives 4–6, which were more soluble in a mixture of toluene and acetonitrile.45 Electrochemical studies with these shorter models 4–6 in such an organic media yet remain relevant to discuss the overall trend among the electrochemical properties of 1–3, as (i) the fullerene cores are expected to lie on the inside of the micellar structures, having an organic media character, and (ii) the differences in electrochemical properties in such a low dielectric phase is expected to mainly arise from inner-sphere reorganization energy differences, which should not be significantly impacted by the length of the PEG tail.

As shown in Figure 7, all three compounds displayed three reversible reduction processes. As summarized in Table 2, the redox potential of the \( \text{C}_{2n-6}^{*-}/\text{C}_{2n}^{**} \) couple is given by the first redox process: among \( \text{C}_{70} \) analogues, compound 6 appears to be reduced at more modest potentials than compound 5. From a thermodynamic point of view, this indicates that 6 has a weaker driving force for part 2 of the type I mechanism (i.e., the generation of \( \text{O}_2^{**} \)) than 5 but also that the reduced fullerene species can be generated in part 1 of the mechanism at a lower energetic cost. This latter point is in good agreement with the higher efficiency and stronger DNA damage enhancement observed for 3 vs 2 in the presence of NADH.

![Figure 7. Cyclic voltammograms of \( \text{C}_{70} \) Prato adducts 4-6 in toluene/acetonitrile (10:4:1) with 0.1 M TBAPF6 at room temperature. The working electrode was a glassy carbon plate, the reference electrode was Ag/AgCl, and the counter electrode was a Pt wire. The scan rate was 100 mV s\(^{-1}\). The second scan is shown.](https://doi.org/10.1021/jacsau.1c00239)

### Table 2. Redox Potentials and Electron Transfer Rates of Prato Adducts 4–6 Obtained from Cyclic Voltammetry\(^a\)

| compd | reduction potentials [V]\(^b\) | electron transfer rate [cm s\(^{-1}\)] |
|-------|-------------------------------|----------------------------------------|
| 4     | \(-1.18\) – \(-1.59\) – \(-2.15\) | \(2.6 \times 10^{-4}\) |
| 5 (ab-[6,6]) | \(-1.18\) – \(-1.58\) – \(-2.04\) | \(1.5 \times 10^{-3}\) |
| 6 (cc-[6,6]) | \(-1.14\) – \(-1.56\) – \(-2.05\) | \(2.6 \times 10^{-3}\) |

\(a\) Obtained from Figure 7. \(b\) In toluene/MeCN (10:4:1) and vs Fc/Fc\(^+\).\n
The reduction of 3 occurs with a higher driving force than the reduction of 2. Interestingly, the observation of a comparable amount of \( \text{O}_2^{**} \) in the type I mechanism with the \( \text{C}_{50} \) compound 1 (e.g., in the presence of \( \text{l}\)-histidine, Figure 5b), despite the more negative redox potential of 4 vs 6, highlights that the driving force of part 2 of the type I mechanism has no direct influence of the efficiency of \( \text{O}_2^{**} \) generation. Hence, only part 1 of the type I mechanism will be considered in the discussion below, in agreement with that step being identified as the rate-determining step by the computational work presented later in text.

However, these thermodynamic considerations do not allow us to fully explain the different behavior of the two \( \text{C}_{70} \) analogues observed above with respect to the type of mechanism considered. Compound 3 operates mainly via a type I mechanism, while compound 2 operates via both type I and U mechanisms occurring in parallel. To get further insight on the generation of \( \text{C}_{70}^{**} \) species and to determine the type of reaction mechanisms involved, we estimated the kinetics for their formation from the cyclic voltammetry data using the method proposed by Magno and co-workers.46 The slope of the \( \psi \) vs \((\text{anodic FD/RT})^{-1/2}\) plot gives the heterogeneous rate constant \(k\) (Figures S31, S33, and S35), where \( \psi \) is defined as

\[
\psi = \frac{(-0.6288 + 0.0021X)}{(1 - 0.017X)}
\]

where \( X \) is the peak-to-peak separation of the anodic and cathodic waves expressed in mV. The electron transfer rate of the \( \text{C}_{70} \) Prato adduct 5 is ca. 1.8 times slower than the ones of \( \text{C}_{60} \) adduct 4 and \( \text{C}_{70} \) adduct 6. The significantly slower reduction rate of 5, with respect to 4 and 6, suggests that the less efficient generation of \( \text{O}_2^{**} \) via a type I energy transfer reaction observed by ESR in the presence of \( \text{l}\)-histidine (Figure 5c) for 2, with respect to 3, results from the slower kinetics for the reduction of 2. Interestingly, this correlates well with the longer lifetime of the \( ^{1}\text{C}_{70} \) species observed for 2, with respect to 3, in agreement with a faster electron transfer rate for 3, contributing to the consumption of the \( ^{1}\text{C}_{70} \) species. These electrochemical studies highlight the interesting predictive potential of simple cyclic voltammetry measurements for the design of efficient fullerene-based compounds for ROS generation. Two favorable scenarios can be identified by electrochemical techniques: for a given \( \text{C}_{70} \) compound family, the compound presenting the most cathodic redox potential will possess a higher driving force for the generation of \( \text{O}_2^{**} \) via a type I energy transfer reaction, while a slow reduction rate will promote the type II reaction mechanism.

### Computational Studies

To further investigate the mechanism of the ROS generation from \( \text{C}_{70}\)-PEG 1–3 through the type I and the type II reactions, computational studies were performed. To make calculations
more feasible, NADH and C$_{60}$-PEG 1–3 were replaced with the model compounds shown in Figure S38. The rate of the studied processes can be estimated using the classical Marcus equation:

$$k_{\text{et}} = \frac{2\pi e^2 |H_{\text{et}}|^2}{\hbar v} \frac{1}{(4\pi k_B T)^{1/2}} \exp \left( -\frac{(\lambda + \Delta G)^2}{4k_B T} \right)$$

where $|H_{\text{et}}|$ is the electronic matrix element describing the electronic coupling between the electronic states of the reactants and the products, $\Delta G$ is the Gibbs energy, and $\lambda$ is the total reorganization energy. $\Delta G$ is the sum of two components, the internal reorganization energy ($\lambda_i$) and the external reorganization energy ($\lambda_e$). $|H_{\text{et}}|$ is known to be quite sensitive to the mutual orientation of the donor and acceptor sites and this dependence makes $|H_{\text{et}}|$ calculation problematic for the studied systems, which are quite flexible. When comparing reaction rates, we have assumed similar $|H_{\text{et}}|$ for species 1-3. For all considered processes, the limit between the normal and the inverted Marcus regime, and consequently the highest predicted rate, is reached with $\Delta G$ (Gibbs energy) = $-\lambda_i$ (reorganization energy). Analyses of ($\Delta G + \lambda_e$) and relative rates for the $\text{type II}$ reaction are summarized in Table 3. Table S1 contains the values of $\Delta G$ and $\lambda_e$. The Gibbs energy of the $\Sigma^g$ (O$_2$) to $^1\Delta_g$ (O$_2$) process was taken from experiments.

### Table 3. Sum of Gibbs Energies and Total Reorganization Energies ($\Delta G + \lambda_e$) and Relative Rates for the $\text{type II}$ Reaction

| systems   | $\Delta G + \lambda_e$ [eV] | relative rate |
|-----------|----------------------------|---------------|
| 1/4 (model) | -0.754                    | slow          |
| 2/5 (model) | -0.368                    | medium/fast   |
| 3/6 (model) | -0.297                    | medium/fast   |

For all the C$_{60}$-PEG models, the $\text{type II}$ reaction occurs in the inverted Marcus regime ($\Delta G < \lambda_e$). By considering that the ISC and the O$_2$ generation process are linked reactions, in which the product of the first reaction becomes the substrate of the second reaction, the rate of the whole process should be determined by the rate of the slowest reaction. The similar computed ($\Delta G + \lambda_e$) values for the $\text{type II}$ mechanism for 2 and 3 further confirm the conclusions of the electro- and photochemical experiments: the $\text{type II}$ mechanism presents a comparable driving force and kinetics for both compounds, but it occurs in competition with the $\text{type I}$ mechanism for both. As the $\text{type I}$ mechanism is disfavored by the slow electron transfer rate for 2, this results in the longer lifetime of the $^1\text{C}_{60}$* state for 2, as observed in the photophysical data and the increased occurrence of the $\text{type I}$ mechanism.

The ($\Delta G + \lambda_e$) values for the $\text{type I}$ reaction mechanism are gathered in Table 4. The $\text{type I}$ reaction involves also the initial ISC, and then it can be divided into two parts (parts 1 and 2, shown in the Scheme of Table 4). To better investigate the role of each process in the determination of the rate of the generation of O$_2$*, we have computed the ($\Delta G + \lambda_e$) and relative rates for parts 1 and 2 of the $\text{type I}$ reaction (Table 4).

### Table 4. Sum of Gibbs Energies and Total Reorganization Energies ($\Delta G + \lambda_e$) and Relative Rates for the $\text{Type I}$ Reaction

| systems | $\Delta G + \lambda_e$ [eV] | $\text{type I, part 1}$ | $\text{type I, part 2}$ |
|---------|---------------------------|-------------------------|-------------------------|
|         |                           | relative rate           | relative rate           |
| 1/4 (model) | 0.132                    | fast                    | 0.323                   |
| 2/5 (model) | 0.379                    | medium                  | 0.540                   |
| 3/6 (model) | 0.416                    | slow/medium            | 0.526                   |

For all the C$_{60}$-PEG models, parts 1 and 2 of the $\text{type I}$ reaction occur in the normal Marcus regime ($\Delta G > \lambda_e$). Hence, the calculated higher reduction rate of C$_{60}$ (part 1) observed for 2 vs 3 is in agreement with the more negative redox potential determined for 2 vs 3. The faster kinetics of compound 3 determined here results from the lower reorganization energy $\lambda_e$ of the C$_{60}$ compound, with respect to the C$_{70}$ ones (Table S1). On the other hand, the final generation of O$_2$* in part 2 show similar rates for all species 1–3. The slightly more efficient generation of O$_2$* by C$_{60}$-PEG 1 measured in the ESR experiments (Figure 5), in spite of its lower absorbance, suggests that part 1 is the rate-limiting step of $\text{Type I}$ reaction ($k_{\text{trapl, part 1}} < k_{\text{trapl, part 2}}$), in agreement with the electrochemical data.

### CONCLUSION

The well-defined water-soluble C$_{60}$-PEG and C$_{70}$-PEG conjugates 1–3 were prepared and tested for photoinduced DNA damage and ROS generation under visible light irradiation. The generation of O$_2$ and photo DNA cleavage via the $\text{type II}$ energy transfer pathway was higher in C$_{70}$-PEG 2 with the 6-[6,6]-addition site. Efficient generation of O$_2$* from C$_{70}$-PEG 2 was explained by (1) the higher absorption intensity at 528 nm and (2) the longer lifetime of its triplet excited state, as suggested by laser flash photolysis measurements. The DNA damage in the presence of electron donor via the $\text{type I}$ electron transfer pathway was much higher in C$_{60}$-PEG 1 despite its lower absorption intensity. The O$_2$* generation, which is partially related to the DNA damage, was observed to be higher in C$_{60}$-PEG 1 and C$_{70}$-PEG 3, especially in the presence of l-histidine. By the cyclic voltammetry measurements, C$_{60}$-PEG 1 and C$_{70}$-PEG 3 were characterized by the same faster electron transfer rates, providing more generation of O$_2$* probably due to its larger impurity diffusion enhancement of interdiffusion (IDEI). Computational results based on the Marcus equation confirm the experimental findings showing that the $\text{type II}$ energy transfer reaction occurs in the inverted Marcus regime whereas the $\text{type I}$ electron transfer reaction proceeds in the normal Marcus regime.

This work showed, for the first time, a clearly different preference of ROS generation from the photoexcited C$_{60}$-PEG 1 and C$_{70}$-PEG 3, generating more O$_2$* from the photoexcited C$_{70}$-PEG 2, generating more O$_2$* in water. The pathway disparity was dependent on the fullerene core (C$_{60}$ or C$_{70}$) and functionalization pattern. The result provides the guidelines for important parameters to consider and optimize
(excited states lifetime, redox potential, electron transfer rate) for the preparation of efficient PDT-PSs. Importantly, but not part of this investigation, type I reaction part 1 represents not only a reduction of $3C_{2n}^*$ but also an oxidation of a nonspecified reaction partner. To understand better the mechanism of DNA damage by ROS generated from photoexcited fullerenes, further investigations using substrates such as guanine or 8-oxoguanine are in progress.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications Web site. The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.1c00239.

Detail of the preparation of C$_{60}$-PEG conjugates with corresponding spectral data, DLS data, experimental methods for DNA cleavage tests and ESR spin-trapping methods; full computational details, full reference, model compounds, Cartesian coordinates of all optimized species; values of Gibbs energies $\Delta G$ and total reorganization energy $\lambda_i$ computed in water for systems 1–3 (PDF)

## AUTHOR INFORMATION

### Corresponding Authors

Yoko Yamakoshi — Laboratorium für Organische Chemie, ETH Zürich, CH-8093 Zürich, Switzerland; Email: yamakoshi@org.chem.ethz.ch

Victor Mougel — Laboratorium für Anorganische Chemie, ETH Zürich, CH-8093 Zürich, Switzerland; Email: mougel@inorg.chem.ethz.ch

Miquel Solà — Institut de Química Computacional i Catàlisi (IQCC) and Departament de Química, Universitat de Girona, 17003 Girona, Catalonia, Spain; Email: miquel.sola@udg.edu

### Authors

Korinne Liosi — Laboratorium für Organische Chemie, ETH Zürich, CH-8093 Zürich, Switzerland

Anton J. Stasyuk — Institut de Química Computacional i Catàlisi (IQCC) and Departament de Química, Universitat de Girona, 17003 Girona, Catalonia, Spain; Email: stasyuk@icp.ub.edu

Fabio Masero — Laboratorium für Anorganische Chemie, ETH Zürich, CH-8093 Zürich, Switzerland

Alexander A. Voityuk — Institut de Química Computacional i Catàlisi (IQCC) and Departament de Química, Universitat de Girona, 17003 Girona, Catalonia, Spain; Instituto Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Catalonia, Spain; Email: a voxelu@icp.ub.edu

Thomas Nauser — Laboratorium für Anorganische Chemie, ETH Zürich, CH-8093 Zürich, Switzerland; Email: thomas.nauser@org.chem.ethz.ch

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/jacsau.1c00239

## Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

The authors thank Dr. Sologubenko in ScopeM at ETH for her help in STEM measurements and analyses. The authors thank Prof. Hilvert in ETH for the access to the transilluminator. The authors thank Prof. Morbidelli and Prof. Arosio in ETH for their help in DLS analysis. The ScopeM of ETH, the NMR service, and MoBiAS in the LOC at ETH are acknowledged for their help in measurements. This study was supported in part by the Swiss National Foundation (200021_15697, 205321_17318, Y.Y.), the ETH Research Grant (ETH-21 15-2, ETH-25 11-I, Y.Y.), the Spanish MINECO (Network RED2018-102815-T, projects CTQ2017-85341-P and PID2020-113711GB-I00, and Juan de la Cierva contract IJC2019-039846-I to A.J.S.) and the Catalan DIVE (2017SGR39).

## REFERENCES

1. Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugira, Y. Photoinduced Biochemical-Activity of Fullerene Carboxylic-Acid. J. Am. Chem. Soc. 1993, 115 (17), 7918–7919.

2. Nakamura, E.; Isobe, H. Functionalized Fullerenes in Water. The First 10 Years of Their Chemistry, Biology, and Nanoscience. Acc. Chem. Res. 2003, 36 (11), 807–815.

3. Castro, E.; Hernandez Garcia, A.; Zavala, G.; Echegoyen, L. Fullerenes in Biology and Medicine. J. Mater. Chem. B 2017, 5 (32), 6523–6535.

4. Arbogast, J. W.; Darmanyan, A. P.; Foote, C. S.; Rubin, Y.; Diederich, F. N.; Alvarez, M. M.; Anz, S. J.; Whetten, R. L. Photophysical Properties of C$_{60}$. J. Phys. Chem. 1991, 95 (1), 11–12.

5. Arbogast, J. W.; Foote, C. S. Photophysical Properties of C$_{70}$. J. Am. Chem. Soc. 1991, 113 (23), 8886–8889.

6. Nagano, T.; Arakane, K.; Ryu, A.; Masunaga, T.; Shinmoto, K.; Mashiko, S.; Hirobe, M. Comparison of Singlet Oxygen Production Efficiency of C$_{60}$ with Other Photosensitizers, Based on 1268-nm Emission. Chem. Pharm. Bull. 1994, 42 (11), 2291–2294.

7. Fraelich, M. R.; Weisman, R. B. Triplet-States of C$_{60}$ and C$_{70}$ in Solution - Long Intrinsic Lifetimes and Energy Pooling. J. Phys. Chem. 1993, 97 (43), 11145–11147.

8. Wasielewski, M. R.; O’Neil, M. P.; Lykke, K. R.; Pellin, M. J.; Gruen, D. M. Triplet-States of Fullerenes C$_{60}$ and C$_{70}$ - Electron-Paramagnetic Resonance-Spectra, Photophysics, and Electronic-Structures. J. Am. Chem. Soc. 1991, 113 (7), 2774–2776.

9. Kumar, R.; Gleissner, E. H.; Tu, E. G. V.; Yamakoshi, Y. C$_{60}$ as a Photocatalyst for Oxidation of Secondary Benzylamines to Imines. Org. Lett. 2016, 18 (2), 184–187.

10. Krusic, P. J.; Wasserman, E.; Parkinson, B. A.; Malone, B.; Holler, E. R.; Keizer, P. N.; Morton, J. R.; Preston, K. F. Electron-Spin-Resonance Study of the Radical Reactivity of C$_{60}$. J. Am. Chem. Soc. 1991, 113 (16), 6274–6275.

11. Arbogast, J. W.; Foote, C. S.; Kao, M. Electron-Transfer to C$_{60}$. J. Am. Chem. Soc. 1992, 114 (6), 2277–2279.

12. Yamakoshi, Y.; Sueyoshi, S.; Fukuhara, K.; Miyata, N.; et al. OH and O$_2$ Generation in Aqueous C$_{60}$ and C$_{70}$ Solutions by Photointradiation: An EPR study. J. Am. Chem. Soc. 1998, 120 (47), 12363–12364.

13. Yamakoshi, Y.; Umezawa, N.; Ryu, A.; Arakane, K.; Miyata, N.; Goda, Y.; Masunizumi, T.; Nagano, T. Active Oxygen Species Generated From Photoexcited Fullerenes (C$_{60}$) as Potential
Defined Amphiphilic C60-PEG Conjugates: Water-Soluble and Correction (DFT-D) for the 94 Elements H-Pu. Design and Assessment of Accuracy. (CAM-B3LYP).

Effects of [70] Fullerene Mono-Adduct Acceptors in Bulk Hetero-

Kurotobi, K.; Higashino, T.; Koganezawa, T.; Tsujimoto, M.; Gelinas, D.; Garcia-Borras, M.; Trapp, N.; Osuna, S.; Yamakoshi, Y. Site-Selective Prato Additions to C70: Experimental and Theoretical Studies of a New Thermodynamic Product at the dd-[5,6]-Junction. Acc. Chem. Res. 2010, 43 (4), 564–571.

Alden Cycloadditions of Butadiene to C70. An Insight into the Chemical Reactivity of C70 as Compared to C60. Photochem. Photobiol. 2013, 115 (15), 3491–3496.

Yamakoshi, Y.; Aroua, S.; Nguyen, T. M.; Iwamoto, Y.; Ohnishi, T. Water-Soluble Fullerenes for Bioapplications: Photoinduced Reactive Oxygen Species Generation. Faraday Discuss. 2014, 173, 287–296.

(14) Yamakoshi, Y.; Aroua, S.; Nguyen, T. M.; Iwamoto, Y.; Meunier, W. H. Chemistry of the Higher Fullerenes - Preparative and Isolation of C76 by HPLC and Synthesis, Separation, and Characterization of Diels-Alder Monoadducts of C70 and C76O2 Fullerene Derivatives. Helv. Chim. Acta 1999, 82 (15), 3297–3304.

(15) Oriana, S.; Aroua, S.; Sollner, J. O.; Ma, X. J.; Iwamoto, Y.; Yamakoshi, Y. Water-soluble C60 and C70-PVP Polymers for Biomaterials with Efficient 1O2 Generation. Biomaterials 2006, 27 (28), 5795–5800.

(16) Iwamoto, Y.; Yamakoshi, Y. A Highly Water-soluble C60-NVP Copolymer: a Potential Material for Photodynamic Therapy. Chem. Commun. 2006, No. 46, 4805–4807.

(17) Sperandio, F. F.; Sharma, S. K.; Wang, M.; Jeon, S.; Huang, Y.-Y.; Dai, T.-H.; Nayka, S.; de Sousa, S. C. O. M.; Chiang, L. Y.; Hamblin, M. R. Photoinduced Electron-Transfer Mechanisms for Radical-Enhanced Photodynamic Therapy Mediated by Water-Soluble Decatacationic C70 and C60O2 Fullerene Derivatives. Nanomedicine 2013, 9 (4), 570–579.

(18) Huang, L.-Y.; Wang, M.; Dai, T.-H.; Sperandio, F. F.; Huang, Y.-Y.; Xuan, Y.; Chiang, L. Y.; Hamblin, M. R. Antimicrobial Photodynamic Therapy with Decatacationic Monoadducts and Bisadducts of [70] Fullerene: in vivo and in vivo Studies. Nanomedicine 2014, 9 (2), 253–266.

(19) Herrmann, A.; Diederich, F.; Thulgen, C.; Termeer, H. U.; Muller, W. H. Chemistry of the Higher Fullerenes - Preparative Isolation of C60 by HPLC and Synthesis, Separation, and Characterization of Diels-Alder Monoadducts of C70 and C76. Helv. Chim. Acta 1994, 77 (7), 1689–1706.

(20) Liosi, K.; Romero-Rivera, A.; Semivrazhskaya, O.; Caniglia, C. D.; Garcia-Borras, M.; Trapp, N.; Osuna, S.; Yamakoshi, Y. Site-Selectivity of Prato Additions to C70: Experimental and Theoretical Studies of a New Thermodynamic Product at the dd-[5,6]-Junction. Org. Lett. 2019, 21 (13), 5162–5166.

(21) Mestres, J.; Duran, M.; Sola, M. Theoretical Study of Diels-

Alder Cycloadditions of Butadiene to C70. An Insight into the Chemical Reactivity of C70 as Compared to C60. J. Phys. Chem. 1996, 100 (18), 7449–7454.

(22) Dallas, P.; Rogers, G.; Reid, B.; Taylor, R. A.; Shinohara, H.; Briggs, G. A. D.; Porphyrakis, K. Charge Separated States and Single Oxygen Generation of Mono and Bis Adducts of C60 and C70. Chem. Phys. 2016, 465, 28–39.

(23) Umeyama, T.; Miyata, T.; Jakowitz, A. C.; Shibata, S.; Kurotobi, K.; Higashino, T.; Koganezawa, T.; Tsujimoto, M.; Gelinas, S.; Matsuda, W.; Seki, S.; Friend, R. H.; Imahori, H. Regioisomer Effect of [70] Fullerene Mono-Adduct Acceptors in Bulk Heterojunction Polymer Solar Cells. Chem. Sci. 2017, 8 (1), 181–188.

(24) Aroua, S.; Tiu, E. G. V.; Ishikawa, T.; Yamakoshi, Y. Well-Defined Amphiphilic C60-PEG Conjugates: Water-Soluble and Thermoresponsive Materials. Helv. Chim. Acta 2016, 99 (10), 805–813.

(25) Aroua, S.; Schweizer, W. B.; Yamakoshi, Y. C60 Pyrrolidino BisCarboxylic Acid Derivative as a Versatile Precursor for Biocompatible Fullerenes. Org. Lett. 2014, 16 (6), 1688–1691.

(26) Yanai, T.; Tew, D. P.; Handy, N. C. A New Hybrid Exchange-Correlation Functional Using the Coulomb-Attenuating Method (CAM-B3LYP). Chem. Phys. Lett. 2004, 393 (1–3), 51–57.

(27) Weigend, F.; Ahlrichs, R. Balanced Basis Sets of Split Valence, Triple Zeta Valence and Quadruple Zeta Valence Quality for H to Rn: Design and Assessment of Accuracy. Phys. Chem. Chem. Phys. 2005, 7 (18), 3297–3308.

(28) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A Consistent and Accurate Ab Initio Parametrization of Density Functional Dispersion Correction (DFT-D) for the 94 Elements H-Pu. J. Chem. Phys. 2010, 132 (15), 154104.

(29) Grimme, S.; Ehrlich, S.; Goerigk, L. Effect of the Damping Function in Dispersion Corrected Density Functional Theory. J. Comput. Chem. 2011, 32 (7), 1456–1465.
(47) Marcus, R. A.; Sutin, N. Electron Transfers in Chemistry and Biology. *Biochim. Biophys. Acta, Rev. Bioenerg.* 1985, 811 (3), 265–322.

(48) Schweitzer, C.; Schmidt, R. Physical Mechanisms of Generation and Deactivation of Singlet Oxygen. *Chem. Rev.* 2003, 103 (5), 1685–757.