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Synthesis and characterization of highly photoresponsive fullereryl dyads with a close chromophore antenna–C₆₀ contact and effective photodynamic potential†

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Abstract

We report the synthesis of a new class of photoresponsive C₆₀–DCE–diphenylaminofluorene nanostructures and their intramolecular photoinduced energy and electron transfer phenomena. Structural modification was made by chemical conversion of the keto group in C₆₀(>DPAF–C_n) to a stronger electron-withdrawing 1,1-dicyanoethylenyl (DCE) unit leading to C₆₀(>CPAF–C_n) with an increased electronic polarization of the molecule. The modification also led to a large bathochromic shift of the major band in visible spectrum giving measureable absorption up to 600 nm and extended the photoresponsive capability of C₆₀–DCE–DPAF nanostructures to longer red wavelengths than C₆₀(>DPAF–C_n). Accordingly, C₆₀(>CPAF–C_n) may allow 2γ-PDT using a light wavelength of 1000–1200 nm for enhanced tissue penetration depth. Production efficiency of singlet oxygen by closely related C₆₀(>DPAF–C_{2M}) was found to be comparable with that of tetraphenylporphyrin photosensitizer. Remarkably, the ¹O₂ quantum yield of C₆₀(>CPAF–C_{2M}) was

†Electronic supplementary information (ESI) available: Fig. S1: ¹H NMR spectrum of C₆₀(>DCE–Ph), Fig. S2: Infrared spectra of (a) C₆₀(>DPAF–C₉) and (b) C₆₀(>CPAF–C₉), Fig. S3: Infrared spectra of (a) C₆₀(>DPAF–C₂) and (b) C₆₀(>CPAF–C₂) for comparison, Fig. S4: ¹H NMR spectrum of C₆₀(>CPAF–C₂), Fig. S5: ¹³C NMR spectra of (a) C₆₀(>CPAF–C₂) and (b) expanded aromatic carbon peaks region, Fig. S6: ¹³C NMR spectra of (a) C₆₀(>DPAF–C₉) 7–C₉ and (b) C₆₀(>CPAF–C₉) 8–C₉, Fig. S7: Negative ion desorption chemical ionization mass spectrum (DCI[–]–MS) of C₆₀(>CPAF–C₂). CCDC reference number 762136. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0jm00037j

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found to be nearly 6-fold higher than that of $C_{60}(>>DPAF-C_{2M})$, demonstrating the large light-harvesting enhancement of the CPAF- C_{2M} moiety and leading to more efficient triplet state generation of the $C_{60}>$ cage moiety. This led to highly effective killing of HeLa cells by $C_{60}(>>CPAF-C_{2M})$ *via* photodynamic therapy (200 J cm⁻² white light). We interpret the phenomena in terms of the contributions by the extended π -conjugation and stronger electron-withdrawing capability associated with the 1,1-dicyanoethylenyl group compared to that of the keto group.

Introduction

Single-photon excitation based photodynamic therapy (1 γ -PDT) for conditions including basal-cell carcinoma, psoriasis, actinic keratosis, oesophageal dysplasia, restenosis and age-related macular degeneration (AMD), in light-accessible organs, such as the digestive tract, artery and eye has achieved success in the past decade.¹ The approach provides an alternative to chemo- and radio-therapeutic treatments of cancer. Effective PDT method requires a combination of a convenient light source and the selection of photosensitizers capable of generating reactive oxygen species (ROS), such as 1O_2 , O_2^- and $\dot{H}O$,² that induce spatially confined cell death in the disease-affected area upon irradiation.

Two-photon excitation based photodynamic therapy³ (2 γ -PDT) has an additional advantage of being able to deliver effective treatment deeper into the tissue at the focal area of irradiation, lessening damage to the healthy adjacent and overlying tissue. However, to make 2 γ -PDT applications efficient, significantly larger two-photon absorption (2PA) cross-sections (σ_2)⁴ of the photosensitizer are required that often involves the specific design of photoresponsive chromophores for this specific purpose.⁵ In addition, the wavelength range of 700–1200 nm generally used in the 2 γ -PDT treatment enhances the penetration depth and fits well with the optical window for biological tissue.

Photoexcitation of C_{60} and its derivatives induces a singlet fullereryl excited state that is transformed to the corresponding triplet excited state, *via* intersystem energy crossing, with nearly quantitative efficiency.⁶ Subsequent energy transfer from the triplet fullerene derivatives to molecular oxygen produces singlet molecular oxygen (1O_2) in aerobic media. This photocatalytic effect is responsible for cytotoxicity involved in anticancer and antimicrobial photodynamic therapy using [60]fullereryl derivatives as photosensitizers,^{7–9}

We previously reported the ultrafast photoresponsive C_{60} -antenna conjugate molecules $C_{60}(>>DPAF-C_n)_x$ ($x = 1, 2$ or 4) that exhibited simultaneous multiphoton excitation events with the observed significant nonlinear photophysical properties.¹⁰ These compounds showed large structure- and concentration-dependent two-photon absorption (2PA) cross-section (σ_2) values and nonlinear laser intensity transmittance capability. For example, at concentrations between 10^{-4} or 10^{-2} M, both $C_{60}(>>DPAF-C_9)_2$ and $C_{60}(>>DPAF-C_9)_4$ exhibited a large fs 2PA cross-section value of 4300 or 80 GM and 8520 or 710 GM, respectively. These values were higher than 2190 or 30 GM, respectively, for the linear $C_{60}(>>DPAF-C_9)$ structure. High two-photon absorption cross-sections were correlated with increased electronic interactions and molecular polarization between the closely linked DPAF- C_n donor antenna and the C_{60} acceptor cage. This facilitated ultrafast energy transfer from photoexcited $C_{60}(>>^1DPAF^*-C_n)$ to the C_{60} moiety forming the excited singlet $^1C_{60}^*(>>DPAF-C_n)$ and subsequently the triplet $^3C_{60}^*(>>DPAF-C_n)$, *via* intersystem crossing. These intramolecular electron and energy events are similar to those occurring with single-photon excitation. One particular application of materials exhibiting nonlinear optical (NLO) effects is the development of efficient light intensity attenuators for sensor development and personnel protection. The feasibility of this goal was demonstrated by the detection of nonlinear optical transmittance reduction responses in the

femtosecond (fs) region with a low transmittance value (35–40%) for $C_{60}(>>DPAF-C_9)_x$ ($x = 1, 2$ or 4) at the laser irradiance intensity level above 600 to 850 GW cm^{-2} .¹⁰

In our continuing efforts to extend the maximum range of the optical absorption of DPAF- C_n moieties (centered at $\sim 400 \text{ nm}$) and their corresponding subsequent fluorescence emissions from $^1DPAF^*-C_n$ state at $450\text{--}460 \text{ nm}$ (in toluene), to longer wave-lengths in the red region for both NLO and PDT, we modified the structure of $C_{60}(>>DPAF-C_n)$ in order to allow light harvesting across a greater visible spectrum. Accordingly, we replaced the keto functional group of C_{60} -keto-DPAF by a highly electron-withdrawing 1,1-dicyanoethylenyl (DCE) bridging group forming C_{60} -DCE-DPAF structures that were analogous to $C_{60}(>>CPAF-C_n)$ derivatives.¹¹ Interestingly, this chemical modification resulted in a large bathochromic shift of ground-state absorption of CPAF- C_n moieties beyond $400\text{--}600 \text{ nm}$. The solution of $C_{60}(>>CPAF-C_n)$ adducts were found to be dark burgundy-red in color. In the molecular structure of new C_{60} -DCE-DPAF assemblies, intramolecular electronic interactions between the 1,1-dicyanoethylenyl bridging group of the CPAF- C_n moiety and the C_{60} cage, *via* through-space periconjugation, are expected to be significantly more active than those of $C_{60}(>>DPAF-C_n)$. This was due to the π -conjugation bonding consideration of both entities and a close inter-space contact of the cyano group to the C_{60} cage in only roughly $2.0\text{--}3.0 \text{ \AA}$ of the separation distance. Upon such structural variation, one particular effect observed upon the change of photophysical properties is the large enhancement of singlet oxygen (1O_2) production efficiency of $C_{60}(>>CPAF-C_n)$ in toluene in comparison with those of $C_{60}(>>DPAF-C_n)$.

In this report, we describe the synthesis, structural characterization, and several photophysical properties of 7-(1,2-dihydro-1,2-methano[60]fullerene-61-{1,1-dicyanoethylenyl})-9,9-diethyl-2-diphenylaminofluorene $C_{60}(>>CPAF-C_2)$ and its related 9,9-di(3,5,5-trimethylhexyl) derivative $C_{60}(>>CPAF-C_9)$, in terms of intramolecular energy and electron transfer processes studied by nanosecond transient measurements. The results were correlated to highly effective killing of HeLa cells under 1γ -PDT conditions.

Materials and methods

General

Reagents 2-bromofluorene, malononitrile, tris(dibenzylideneacetone) dipalladium(0) and *rac*-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) were purchased from Aldrich Chemicals. All other chemicals and solvents, including toluene (TN), *o*-dichlorobenzene (*o*-DCB), benzonitrile (PhCN) and CS_2 were purchased from Acros Ltd. Pure C_{60} (99.5%) was purchased from NeoTech Product Company, Russia and used as received.

UV-vis spectra were recorded on either a Hitachi U-3410 UV spectrometer or Hewlett-Packard diode-array model 8452A UV-visible spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer LS55 luminescence spectrometer. Infrared spectra were recorded as KBr pellets on a Nicolet 750 series FT-IR spectrometer. ^1NMR , ^{13}C NMR and COSY spectra were recorded on either a Bruker Avance Spectrospin-400 or Bruker AC-300 spectrometer. HMQC spectra were recorded on Bruker-500 SB FT-NMR spectrometer. Mass spectroscopic measurements were performed by the use of positive ion matrix-assisted laser desorption ionization (MALDI-TOF) technique on a micromass M@LDI-LR mass spectrometer. Negative ion desorption chemical ionization (DCI $^-$) mass spectra were collected using a direct probe on a JEOL JMS-SX 102A mass spectrometer. Positive ion fast atom bombardment (FAB $^+$) mass spectra were collected using a direct probe on a JEOL SX-102A mass spectrometer. Elemental analyses of fullerene derivatives and X-ray single crystal analysis, performed on NONIUS KAPPA CCD diffractometer, were carried out at National Instrumentation Center located at National Taiwan University, Taiwan.

Synthesis of (1,2-methano[60]fullerene-61-carbonyl)benzene, $C_{60}(>>CO-Ph)$, **1**

To the mixture of C_{60} (1.0 g 1.38 mmol) and phenacyl bromide (276 mg, 1.38 mmol) in toluene (700 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.210 mL, 1.41 mmol) under nitrogen atmosphere. After stirring at room temperature for a period of 5 h, the reaction mixture was filtered and concentrated to a 10% volume. Crude products were precipitated by the addition of methanol. The precipitates were isolated by centrifugation and purified by column chromatography (silica gel, hexane–toluene (8:2)). A chromatographic band corresponding to $R_f = \sim 0.4$ on an analytical thin-layer chromatographic plate (TLC, hexane–toluene (8:2)) was collected to afford (1,2-methano[60]fullerene-61-carbonyl)benzene **1**, $C_{60}(>>CO-Ph)$, as a brown solid (450 mg) in 52% yield (based on recovered C_{60}); DCI⁺-MS: calc. for $^{12}C_{68}^{16}O_1$ m/z 838; found, 838.0. UV-vis ($CHCl_3$, 2.0×10^{-5} M) λ_{max}/nm ($\epsilon/L\ mol^{-1}\ cm^{-1}$): 260 (1.2×10^5) and 327 (3.9×10^4).

Synthesis of the model compound (1,2-methano[60]fullerene-61-{1,1-dicyanoethylene})benzene, $C_{60}(>>DCE-Ph)$, **2**

(1,2-Methano[60]fullerene-61-carbonyl)benzene **1**, $C_{60}(>>CO-Ph)$, (500 mg, 0.6 mmol) and malononitrile (78 mg, 1.2 mmol) were dissolved in dry toluene (~ 250 mL) under nitrogen atmosphere. Pyridine (188 mg, 0.2 mL, 2.4 mmol) was added to the solution with stirring. To the resulting mixture was then added titanium tetrachloride (560 mg) in an excess. After stirring at room temperature for a period of 5 min, the reaction mixture was quenched with water (100 mL). The organic layer was washed several times with water, dried over magnesium sulfate, and concentrated in vacuum to afford orange–red solids. Crude products were purified by preparative thin-layer chromatography (TLC, silica gel, hexane–toluene (3:2)). A chromatographic band corresponding to $R_f = \sim 0.4$ on analytical TLC (hexane–toluene (8:2)) was collected to afford (1,2-methano[60]fullerene-61-{1,1-dicyanoethylene})benzene **2**, $C_{60}(>>DCE-Ph)$, as orange–brown solids (340 mg) in 66% yield; DCI⁺-MS: calc. for $^{12}C_{71}^{16}N_2$ m/z 886; found, 886.0; UV-vis ($CHCl_3$, 2.0×10^{-5} M) λ_{max}/nm ($\epsilon/L\ mol^{-1}\ cm^{-1}$): 260 (1.3×10^5) and 326 (5.2×10^4); 1H NMR (400 MHz, $CDCl_3$, ppm) δ 8.07 (m, 2H), 7.61 (m, 3H) and 5.48 (s, α -proton, 1H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm) δ 169.0, 147.3, 145.5, 145.4, 145.3 ($\times 3$), 145.1, 144.9, 144.8, 144.7, 144.6, 143.8, 143.0 ($\times 2$), 142.4, 142.1 ($\times 2$), 142.0 ($\times 2$), 141.2, 137.4, 137.0, 134.7, 133.5, 129.7, 128.4, 112.6 ($C\equiv N$), 112.5 ($C\equiv N$), 90.4, 72.2 (C_{F1} , C_{F2}) and 41.0 (C_{61}).

Synthesis of 7-[1-(1,1-dicyanoethylene)-2-bromoethyl]-9,9-diethyl-2-diphenylaminofluorene **6-C₂** (BrCPAF-C₂)

In a reaction flask, 7-bromoacetyl-9,9-diethyl-2-diphenylaminofluorene **5-C₂** (BrDPAF-C₂, 150 mg, 0.3 mmol) and malononitrile (38 mg, 0.6 mmol) were added followed by the addition of dry toluene (25 mL) under nitrogen atmospheric pressure to give a clear solution. Pyridine (92 mg, 1.2 mmol) and an excess amount of titanium tetrachloride were then added with stirring. After keeping for a period of 5.0 min at ambient temperature, the reaction mixture was quenched with water (30 mL). The resulting organic layer was washed several times with water, dried over magnesium sulfate, and concentrated in *vacuo* to give dark bright red solids. The crude product was purified by preparative thin-layer chromatography (TLC, silica gel) using a solvent mixture of chloroform–hexane (1:1) as eluent. A chromatographic fraction, corresponding to R_f 0.6 on an analytical TLC plate using the same eluent, was isolated to afford 7-[1-(1,1-dicyanoethylene)-2-bromoethyl]-9,9-diethyl-2-diphenylaminofluorene **6-C₂** (BrCPAF-C₂) as dark red solids in a yield of 67% (110 mg).

Spectroscopic data for **5-C₂**: FAB⁺-MS: calc. for $^{12}C_{31}^{128}^{79.9}Br_1^{14}N_1^{16}O_1$ m/z 510; found, m/z 509 and 511; Anal. Calc. for $C_{31}H_{28}BrNO$: C, 72.94; H, 5.49; N, 2.74. Found: C, 73.22; H, 5.62; N, 2.54%. UV-vis ($CHCl_3$, 2.0×10^{-5} M) λ_{max}/nm ($\epsilon/L\ mol^{-1}\ cm^{-1}$): 299 (2.4×10^4) and 406 (2.7×10^4); FT-IR (KBr) ν_{max} 3037 (w), 2966 (s), 2928 (m), 2878 (w), 1674 (s),

1595 (vs), 1491 (s), 1281 (vs), 754 (s) and 698 (s) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3 , ppm) δ 7.95 (dd, $J = 8$ Hz, $J = 1.6$ Hz, 1H), 7.92 (d, $J = 1.4$ Hz, 1H), 7.65 (d, $J = 8$ Hz, 1H), 7.60 (d, $J = 8$ Hz, 1H), 7.28–7.09 (m, 10H), 7.05–7.02 (m, 2H), 4.49 (s, 2H), 2.05–1.84 (m, 4H) and 0.35 (t, $J = 7.3$ Hz, 6H); ^{13}C NMR (400 MHz, CDCl_3 , ppm) δ 191.0, 152.8, 150.3, 148.9, 147.3, 134.3, 131.6, 129.3, 129.2, 128.9, 124.4, 123.1, 122.8, 121.6, 118.8, 118.1, 56.2, 32.4, 31.2 and 8.5.

Spectroscopic data for **6-C₂**: Anal. Calc. for $\text{C}_{34}\text{H}_{28}\text{BrN}_3$: C, 73.71; H, 5.01; N, 7.52. Found: C, 74.03; H, 5.47; N, 6.91%. FAB⁺-MS: calc. for $^{12}\text{C}_{34}^{128}^{79.9}\text{Br}_1^{14}\text{N}_3$ m/z 558; found, m/z 557 and 559; UV-vis (CHCl_3) $\lambda_{\text{max}}/\text{nm}$ ($\epsilon/\text{L mol}^{-1} \text{cm}^{-1}$): 316 (2.8×10^4) and 492 (1.8×10^4); FT-IR (KBr) ν_{max} 3031 (w), 2955 (s), 2921 (s), 2873 (m), 2846 (m), 2225 ($\text{C}\equiv\text{N}$), 1592 (vs), 1541 (m), 1489 (vs), 1463 (s), 1344 (s), 1313 (m), 1280 (vs), 817 (s), 752 (s) and 691 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3 , ppm) δ 7.69 (d, $J = 8$ Hz, 1H), 7.63 (d, $J = 1.6$ Hz, 1H), 7.62 (d, $J = 8$ Hz, 1H), 7.57 (d, $J = 8$ Hz, 1H), 7.28–7.23 (m, 4H), 7.12–7.10 (m, 4H), 7.06–7.01 (m, 4H), 4.59 (s, 2H), 2.00–1.85 (m, 4H) and 0.35 (t, $J = 7.2$ Hz, 6H). ^{13}C NMR (400 MHz, CDCl_3 , ppm) δ 171.1, 152.7, 150.8, 149.3, 147.6, 147.1, 133.8, 131.9, 130.1, 129.4, 127.5, 124.7, 123.4, 122.9, 122.7, 121.7, 119.5, 117.8, 113.2, 112.4, 84.1, 56.5, 32.4, 28.7 and 8.5.

General procedure of the fullerene adduct preparation

Synthesis of 7-(1,2-dihydro-1,2-methanofullerene[60]-61-carbonyl)-9,9-dialkyl-2-diphenylaminofluorene monoadduct 7-C_n, C₆₀(>DPAF-C_n)—Preparative procedures for the synthesis of 7-bromoacetyl-9,9-di(3,5,5-trimethylhexyl)-2-diphenylaminofluorene **5-C₉** (BrDPAF-C₉) follow those reported previously.¹² The reagent compound **5-C₉** (0.97 g, 1.38 mmol) and C₆₀ (1.0 g, 1.38 mmol) were dissolved in toluene (700 mL) under an atmospheric pressure of nitrogen. To this mixture was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.2 mL, 1.38 mmol) and stirred at room temperature for a period of 5 h. At the end of stirring, suspended solids of the reaction mixture were filtered off and the filtrate was concentrated to a 10% volume. Methanol (100 mL) was then added to the liquid to cause precipitation of the crude product, which was isolated by centrifugation. Isolation of the monoadduct **7-C₉** was made by column chromatography (silica gel) using a solvent mixture of hexane–toluene (3:2) as eluent. The chromatographic band at R_f 0.45 on the thin-layer chromatographic (TLC, SiO_2) plate using hexane–toluene (3:2) as eluent afforded C₆₀(>DPAF-C₉) **7-C₉**, 7-(1,2-dihydro-1,2-methanofullerene[60]-61-carbonyl)-9,9-di(3,5,5-trimethylhexyl)-2-diphenylaminofluorene, as greenish brown solids (960 mg, 70% yield based on recovered C₆₀).

Spectroscopic data for C₆₀(>DPAF-C₂) **7-C₂**: Anal. Calc. for $\text{C}_{91}\text{H}_{27}\text{NO}$: C, 95.03; H, 2.34; N, 1.2. Found: C, 94.58; H, 2.63; N, 1.00%. FAB⁺-MS: calc. for $^{12}\text{C}_{91}^{127}^{14}\text{N}_1^{16}\text{O}_1$ m/z 1149; found, m/z 1150; DCI⁺-MS: calc. for $^{12}\text{C}_{91}^{127}^{14}\text{N}_1^{16}\text{O}_1$ m/z 1149; found, m/z 1149; UV-vis (CHCl_3 , 2.0×10^{-5} M) $\lambda_{\text{max}}/\text{nm}$ ($\epsilon/\text{L mol}^{-1} \text{cm}^{-1}$): 260 (1.5×10^5), 326 (6.5×10^4) and 410 nm (4.8×10^4); FT-IR (KBr) ν_{max} 3029 (w), 2963 (s), 2921 (m), 2875 (w), 2853 (w), 1677 ($\text{C}=\text{O}$, s), 1591 (vs), 1492 (s), 1276 (s), 750 (s), 695 (s) and 524 (s) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3 , ppm) δ 8.48 (dd, $J = 8$ Hz, $J = 1.6$ Hz, 1H), 8.32 (d, $J = 1.6$ Hz, 1H), 7.83 (d, $J = 8$ Hz, 1H), 7.66 (d, $J = 8$ Hz, 1H), 7.29–7.11 (m, 10H), 7.07–7.03 (m, 2H), 5.69 (s, 1H), 2.13–1.89 (m, 4H) and 0.40 (t, $J = 8$ Hz, 6H); ^{13}C NMR (400 MHz, CDCl_3 , ppm) δ 189.6 ($\text{C}=\text{O}$), 153.0, 150.8, 149.1, 148.2, 147.9, 147.6, 146.9, 145.6, 145.4, 145.3, 145.1, 145.0, 144.9, 144.7, 144.6 ($\times 2$), 144.3, 143.9, 143.7, 143.3, 143.1, 143.0 ($\times 2$), 142.9, 142.8, 142.5, 142.2 ($\times 2$), 142.1, 141.2, 140.9, 139.7, 136.6, 134.2, 133.5, 129.3, 129.0, 124.5, 123.2, 123.1, 122.8, 121.8, 119.2, 118.1, 72.7 ($\times 2$, C_{F1}, C_{F2}), 56.4, 44.4 (C₆₁), 32.5 and 8.7.

General synthetic procedure of 7-(1,2-dihydro-1,2-methanofullerene[60]-61-(1,1-dicyanoethylene))-9,9-dialkyl-2-diphenylaminofluorene monoadduct 8-C_n, C₆₀(>CPAF-C_n)

Method A—In a reaction flask, to a mixture of 7-(1,2-dihydro-1,2-methanofullerene[60]-61-carbonyl)-9,9-diethyl-2-diphenylaminofluorene C₆₀(>DPAF-C₂) (**7-C₂**, 300 mg, 0.26 mmol) and malononitrile (34 mg, 0.52 mmol) dissolved in dry toluene (25 ml) under nitrogen atmosphere was added pyridine (82 mg, 1.04 mmol) with stirring. An excess amount of titanium tetra-chloride was then added. After stirring for a period of 5.0 min at ambient temperature, the reaction mixture was quenched with water (30 mL). The organic layer was subsequently washed several times with water, dried over magnesium sulfate, and concentrated *in vacuo* to afford orange–red solids. This crude product was purified by preparative thin-layer chromatography (TLC, silica gel) using a solvent mixture of hexane–toluene (3:2) as eluent. A chromatographic fraction, corresponding to *R_f* 0.3 on an analytical TLC plate using the same eluent, was isolated to give 7-(1,2-dihydro-1,2-methanofullerene[60]-61-{1,1-dicyanoethylene})-9,9-diethyl-2-diphenylaminofluorene **8-C₂**, C₆₀(>CPAF-C₂), as orange–red solids in 67% yield (210 mg).

Method B—To C₆₀ (1.0 g, 1.38 mmol) dissolved in dry toluene (700 mL) was added 7-[1-(1,1-dicyanoethylene)-2-bromoethyl]-9,9-diethyl-2-diphenylaminofluorene **6-C₂** (BrCPAF-C₂, 770 mg, 1.38 mmol) in dry toluene (30 mL) under nitrogen. 1,8-Dia-zabicyclo[5.4.0]undec-7-ene (DBU, 0.205 mL, 1.38 mmol) was then added to this mixture. After stirring for a period of 5 h at room temperature, the reaction mixture was filtered and concentrated to a 10% volume. The crude product was precipitated by the addition of methanol and isolated by centrifugation. The solid was further purified by column chromatography (silica gel, hexane–toluene (3:2) as eluent). A chromatographic fraction, corresponding to *R_f* 0.3 on an analytical TLC plate using the same eluent, was isolated to afford 7-(1,2-dihydro-1,2-methanofullerene[60]-61-{1,1-dicyanoethylene})-9,9-diethyl-2-diphenylaminofluorene **8-C₂**, C₆₀(>CPAF-C₂), as orange–red solids in 52% yield (870 mg) or 72% based on recovered C₆₀.

Spectroscopic data for C₆₀(>CPAF-C₂) **8-C₂**: Anal. Calc. for C₉₄H₂₇N₃: C, 94.23; H, 2.25; N, 3.50. Found: C, 94.09; H, 2.55; N, 3.49%; FAB⁺-MS: calc. for ¹²C₉₄¹²⁷¹⁴N₃ *m/z* 1197; found, 1198 (MH⁺), 1197 (M⁺), 768, 745, 720 (C₆₀), 696, 672 and 613; DCl⁺-MS 1197 (M⁺), 900, 730 (C₆₀) and 479 (MH₂⁺-C₆₀); UV-vis (CHCl₃, 2.0 × 10⁻⁵ M) λ_{max}/nm (ε/L mol⁻¹ cm⁻¹): 260 (1.7 × 10⁵), 327 (8.2 × 10⁴), 503 (2.9 × 10⁴); λ_{max} (DMF, ε) 261 (1.2 × 10⁵), 326 (4.4 × 10⁴) and 471 (5.9 × 10³); FT-IR (KBr) ν_{max} 3033 (w), 2963 (s), 2923 (m), 2875 (w), 2853 (w), 2224 (C≡N), 1592 (s), 1492 (s), 1278 (s), 750 (s), 697 (s) and 524 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃, ppm) δ 8.11 (dd, *J* = 8 Hz, *J* = 1.2 Hz, 1H), 7.99 (d, *J* = 1.6 Hz, 1H), 7.78 (d, *J* = 8 Hz, 1H), 7.58 (d, *J* = 8 Hz, 1H), 7.27–2.08 (m, 10H), 7.03–7.00 (m, 2H), 5.52 (s, 1H), 2.05–1.88 (m, 4H) and 0.29 (t, *J* = 7.2 Hz, 6H); ¹³C NMR (400MHz, CDCl₃, ppm) δ 168.8, 152.7, 150.9, 149.4, 147.5, 147.4, 145.8, 145.4, 145.3, 145.2, 145.1, 144.8 (×2), 144.7, 144.5, 144.3, 143.8 (×2), 143.0, 142.4, 142.0 (×2), 141.4, 141.0, 137.5, 137.1, 133.8, 132.2, 129.4, 128.2, 124.6, 123.4, 123.0, 122.7, 121.9, 119.8, 117.7, 113.3 (×2, C≡N), 87.7, 72.5 (×2, C_{F1}, C_{F2}), 56.6, 41.3 (C₆₁), 32.6 and 8.6.

Spectroscopic data for C₆₀(>CPAF-C₉) **8-C₉**: Anal. Calc. for C₁₀₈H₅₅N₃: C, 93.04; H, 3.95; N, 3.02. Found: C, 93.13; H, 3.92; N, 2.82; FAB⁺-MS: calc. for ¹²C₁₀₈¹⁵⁵¹⁴N₃ *m/z* 1393; found, 1395 (MH₂⁺), 1394 (MH⁺), 1155, 1141, 1128, 768, 745, 720 (C₆₀) and 696; UV-vis (CHCl₃, 2.0 × 10⁻⁵ M) λ_{max}/nm (ε/L mol⁻¹ cm⁻¹): 261 (1.3 × 10⁵), 326 (5.3 × 10⁴) and 499 nm (2.0 × 10⁴); FT-IR (KBr) ν_{max} 3029 (w), 2946 (s), 2860 (m), 2222 (C≡N), 1591 (vs), 1490 (s), 1464 (s), 1425 (s), 1277 (s), 1184 (s), 751 (s), 694 (s) and 525 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃, ppm) δ 8.13 (d, *J* = 8 Hz, 1H), 8.03 (t, 1H), 7.75 (d, *J* = 8 Hz, 1H), 7.59 (d, *J* = 8 Hz, 1H), 7.28–7.01 (m, 12H), 5.54 (s, *J* = 4 Hz, 1H), 2.1–1.8 (m, 4H) and 1.28–0.4 (m, 34H). ¹³C NMR (500 MHz, CS₂-CDCl₃, ppm) δ 168.0, 154.0, 152.0, 149.8, 148.0, 147.8, 147.4, 146.5,

146.4, 145.9, 145.8, 145.7, 145.3, 145.1, 145.0, 144.8, 144.3, 144.2 ($\times 2$), 143.5, 143.0, 142.6, 142.5, 141.9, 141.6 ($\times 2$), 137.9, 137.8, 137.5, 133.9, 132.6, 129.9, 128.7, 125.3 ($\times 2$), 125.2, 124.0, 123.2, 122.4, 122.3, 120.3, 118.0, 117.9, 113.6 ($\text{C}\equiv\text{N}$), 113.5 ($\text{C}\equiv\text{N}$), 88.5, 73.0 ($\times 2$), CF_1 , CF_2), 55.8, 51.5, 51.2 ($\times 2$), 42.0 (C_{61}), 33.7, 31.2, 30.6, 30.4, 30.5, 30.0 and 23.1.

Crystal data for 8-C₂—Empirical formula, $\text{C}_{98.25}\text{H}_{36}\text{N}_3\text{O}_{1.5}\text{S}_{0.50}$; formula weight, 1298.33; crystal size $0.25 \times 0.20 \times 0.15$ mm; crystal system, monoclinic; space group, $P2_1/c$; $T = 295(2)$ K; unit cell dimensions $a = 22.0080(2)$, $b = 9.9830(10)$, $c = 28.1780(3)$ Å, $\beta = 93.4330(10)^\circ$; $V = 6179.76(11)$ Å³ with $Z = 4$; $D_c = 1.385$ Mg m⁻³; independent reflections = 10878 ($R_{\text{int}} = 0.0636$); reflections collected = 34057; $R_1 = 0.0831$ and $wR_2 = 0.1932$ for $I > 2\sigma(I)$, $R_1 = 0.1488$ and $wR_2 = 0.2336$ for all data.

Physical measurements

Optical and electrochemical instrumentation—Optical absorption and transmission spectra in correlation to the transient measurements in the range of 180–1200 nm were recorded using a 1-mm thick quartz sample cuvette on a Model UV 3600 UV-vis-NIR spectrophotometer (Shimadzu, Kyoto, Japan).

Electrochemical redox potential values were measured using a BAS CV-50W Voltammetric Analyzer. A conventional three-electrode system was used with a platinum disk electrode (1 mm in diameter) serving as the working electrode with a platinum wire and an Ag/AgCl electrode serving as the counter and reference electrodes, respectively. All measurements were carried out in deaerated benzonitrile and *o*-dichlorobenzene containing tetrabutylammonium perchlorate [$(n\text{-Bu})_4\text{N}^+\text{ClO}_4^-$] (0.1 M) as a supporting electrolyte in a scan rate of 0.1 V s^{-1} . The potentials were referenced to a ferrocene/ferrocenium (Fc/Fc^+) redox couple as an internal standard.

Emission and nanosecond transient absorption measurements—Emission spectra were measured using a Perkin-Elmer model LS 50B fluorometer. Time-correlated single-photon counting (Edinburgh Instruments OB 920 Spectrometer) was utilized to determine singlet state lifetimes (data given in Table 1). The sample was pumped with a 70-ps laser diode at 401 nm. Emission was detected on a cooled microchannel plate PMT. Data were analyzed by using a reconvolution software package provided by Edinburgh Instruments. Nanosecond transient absorption measurements were carried out using 486 nm of a 355 nm optical parametric oscillator (OPO) of a Q-switched Nd:YAG laser (Quantel Brilliant, pulse width *ca.* 5 ns). Pulse fluences of up to 4 mJ cm^{-2} at the excitation wavelength were typically used.

Time-resolved singlet oxygen production measurements—Time-resolved singlet oxygen measurements were performed by direct detection of the near-IR luminescence emission of oxygen ($^1\Delta_g \rightarrow ^3\Sigma_g$ transition) at 1270 nm corresponding to a singlet–triplet transition state. Both the fullerene derivatives were excited by a frequency-doubled Nd:YLF laser (QG-523-500; Crystalaser Inc, Reno, NV) at 523 nm (λ_{max}). The pulse duration was 10 ns and the pulse repetition rate was approximately 3.0 kHz. The singlet oxygen luminescence was detected by a PMT detector (R5509-42; Hamamatsu Corp, Bridgewater, NJ) with high sensitivity in the near-IR region. Five bandpass filters (1210, 1240, 1270, 1300 and 1330 nm) were placed sequentially in front of the photodetector to sample the luminescence spectrum. All measurements were performed using 10 or 50 μM concentrations of the fullerene derivatives in toluene. The time-resolved kinetics of the singlet oxygen luminescence in terms of the concentration of $^1\text{O}_2$ at time t , $S(t)$ were expressed and fitted with the following equation.

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$$S(t) = C[\exp(-t/\tau_t) - \exp(-t/\tau_s)] \quad (1)$$

where C is a constant, τ_t and τ_s are triplet state and singlet oxygen lifetime (ms), respectively.

Cell culture—A human cervical cancer cell line, HeLa was obtained from ATCC (Manassas, VA). The cells were cultured in RPMI medium with L-glutamine and NaHCO_3 supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U mL^{-1}) (Sigma, St. Louis, MO) at 37°C in 5% CO_2 -humidified atmosphere in flasks (75 cm^2 , Falcon, Invitrogen, Carlsbad, CA). When the cell reached 80% confluence, they were washed with phosphate-buffered saline (PBS) and harvested with the trypsin-EDTA solution (0.25%, 2.0 mL, Sigma). Cells were then centrifuged and counted in trypan blue to ensure viability and plated at a density of 5000/well in flat-bottom 96-well plates (Fisher Scientific, Pittsburgh, PA).

In vitro PDT studies—We used a broad-band white-light source (400–700 nm band pass filter, LumaCare, Newport Beach, CA) to deliver 200 J cm^{-2} at an irradiance of 100 mW cm^{-2} as measured with a power meter (Model DMM 199 with 201 Standard head, Coherent, Santa Clara, CA). Illumination took 33 min to complete.

Fullerenyl monoadduct derivatives were dissolved in dimethylacetamide (DMA) to form stock solutions of 5.0 mM . After cells had grown for 24 h, dilutions of fullerene derivatives were prepared in complete RPMI medium containing 10% serum, penicillin (100 U/mL), and streptomycin ($100 \mu\text{g mL}^{-1}$). Fullerene derivatives were added to the cells at 1.0 – $5.0 \mu\text{M}$ concentrations for an additional 3.0 h incubation. The highest DMA concentration in the medium did not exceed 0.1%. The medium was replaced with fresh complete medium and illumination (200 J cm^{-2}) with white light was delivered. The light spot covered four wells, which were considered as one experimental group illuminated at the same time. Control groups were as follows: no treatment, light alone and fullerene derivative alone in the dark (at the same dilutions used for PDT). Following PDT treatment, the cells were returned to the incubator overnight and a 4-h MTT assay was carried out the next day and read at 562 nm using a microplate spectrophotometer (Spectra Max 340 PC, Molecular Devices, Sunnyvale, CA). Each experiment was repeated 3 times.

Results and discussion

Each chemical functionalization by alkyl or aryl addition to a fullerenyl olefin effectively changes the orbital configuration of two linking fullerenyl carbons from sp^2 to sp^3 and makes the direct π -conjugation between the C_{60} cage and attached organic chromophores impossible. Our recent approach to circumventing this issue and enhancing the electronic interactions of light-harvesting photoactive donor antenna with π -orbitals of the C_{60} cage was to apply a bridging π -functional group for connecting these two moieties.¹⁴ This provides the possibility of an increasing amount of partial to full periconjugation. The first bridging functional group used for this purpose was a keto group in the molecular construction of C_{60} -keto-DPAF- C_n assemblies, where DPAF- C_n represents 9,9-dialkyl-2-diphenylaminofluorene. As an example, the solutions of C_{60} (>DPAF- C_n) homologs were found to show large enhancements of two-photon absorption (2PA) cross sections in the femtosecond region as compared to the sum of the values of individual chromophore components.^{10,15} These nonlinear photonic responsive data together with the nanosecond transient absorption measurements based on the linear photoexcitation of the same series of fullerenyl adducts provided strong evidence of the effectiveness of through-space periconjugation interactions. These interactions were proposed to facilitate the high efficiency of intramolecular electron and energy transfer processes from the DPAF- C_n donor antenna to the C_{60} acceptor cage upon photoexcitation.^{16,17} In this study,

we further extended our investigation of the periconjugation approach by functional conversion of $C_{60}(>>DPAF-C_n)$ dyad materials to produce molecules with broadband absorption characteristics.

Accordingly, we carried out chemical modification of the keto group to the 1,1-dicyanoethylenyl (DCE) group leading to the resulting $C_{60}-DCE-DPAF-C_n$ assemblies, $C_{60}(>>CPAF-C_n)$. The higher electron-withdrawing ability of the DCE group compared with the keto group should increase the degree of molecular polarization with negative charges being localized in the region of the fullerene cage. The modification was also expected to decrease the through-space contact distance between the C_{60} cage and the bridging DCE functional group. Owing to a limiting space available in the region, this should result in closer and more effective π -electron interactions between these two moieties.

Synthesis and spectroscopic characterization of $C_{60}(>>CPAF-C_n)$

Chemical modification of a keto group to a DCE group can be made by using malononitrile as a reagent. However, a close contact of DCE group with the C_{60} cage could also be accompanied by the possibility of high steric hindrance at the intermediate reaction center when bonding DCE in a limited space adjacent to the C_{60} . Synthetically, a recently reported reaction sequence for the preparation of 7-bromoacetyl-9,9-dialkyl-2-diphenylaminofluorene **5**- C_n , BrDPAF- C_n , was applied as the key intermediate precursor molecule,¹² as shown in Scheme 1. Addition of a DPAF- C_n subunit to C_{60} leading to the formation of dyads **7**- C_2 , $C_{60}(>>DPAF-C_2)$ and **7**- C_9 , $C_{60}(>>DPAF-C_9)$, was accomplished by the treatment of α -bromoacetylfluorene derivative **5**- C_2 and **5**- C_9 , respectively, with C_{60} in toluene in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 1.0 equiv.) at ambient temperature for 5.0 h. By the control of an equivalent quantity of DPAF- C_n vs. C_{60} , the yield of the monoadduct was maximized with the bisadduct in a low yield and other higher adducts in an insignificant amount.

Conversion of the keto group of **7**- C_2 and **7**- C_9 to the corresponding 1,1-dicyanoethylenyl group was effected by the reaction using malononitrile as a reagent, pyridine as a base, and titanium tetrachloride as a deoxygenation agent in dry toluene at ambient temperature for a short period of 5 min. The reaction afforded 7-(1,2-dihydro-1,2-methanofullerene[60]-61{1,1-dicyanoethylene})-9,9-diethyl-2-diphenylaminofluorene **8**- C_2 , $C_{60}(>>CPAF-C_2)$, as orange-red solids in 67% yield, based on recovered C_{60} , after purification. Alternatively, $C_{60}(>>CPAF-C_2)$ can be synthesized from the reaction of C_{60} with 7-[1-(1,1-dicyanoethylene)-2-bromoethyl]-9,9-diethyl-2-diphenylamino-fluorene **6**- C_2 , BrCPAF- C_2 , in dry toluene in the presence of DBU (1.0 equiv.) at ambient temperature for a period of 5.0 h, as shown in Scheme 1. The reaction resulted in the identical products in a slightly higher yield of 52 or 72% based on recovered C_{60} .

Evidence of the CPAF moiety attached to a methano[60]fullerene cage by a 1,1-dicyanoethylenyl bridging group can be seen clearly from the infrared spectra of **8**- C_2 and **8**- C_9 where both showed a strong absorption band of cyano ($-C\equiv N$) stretching vibration centered at 2222–2224 cm^{-1} with the complete disappearance of carbonyl stretching vibration at 1677 cm^{-1} for both **7**- C_2 and **7**- C_9 . There were also three typical fullerenyl signals at 751, 694 and 525 cm^{-1} for the unfunctionalized half-cage sphere of C_{60} . The former cyano absorption band matches well with that of the model compound $C_{60}(>>DCE-Ph)$ **2** at 2224 cm^{-1} and the BrCPAF- C_2 precursor, at 2225 cm^{-1} , confirming successful conversion of the keto to corresponding DCE moiety. Interestingly, this chemical modification led to a large bathochromic shift of the long-wavelength absorption band of **8**- C_2 to 503 nm ($\epsilon = 2.9 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$) in CHCl_3 ($2.0 \times 10^{-5} \text{ M}$) which was red-shifted nearly 93 nm compared to that of $C_{60}(>>DPAF-C_2)$ (**7**- C_2) or $C_{60}(>>DPAF-C_9)$ (**7**- C_9) centered at 410 nm (λ_{max} , Fig. 1d), as shown in UV-vis spectra of Fig. 1. This band was accompanied with two other absorption bands with λ_{max} centered at 260 ($\epsilon = 1.7 \times 10^5$) and 327 ($\epsilon = 8.2 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$), matching approximately with those

of the model compound $C_{60}(>>CO-Ph)$ (Fig. 1a) containing mainly an optically active fullerene moiety. The absorption profiles also agree well with those of allowed ${}^1T_{1u} \rightarrow {}^1A_g$ transition bands of pristine C_{60} .¹⁸ The comparison provided the confirmation of these two absorption bands being attributed to the C_{60} cage. Similarly, the compound **8**-C₉ displayed three absorption bands with λ_{max} centered at 261 ($\epsilon = 1.3 \times 10^5$), 326 ($\epsilon = 5.3 \times 10^4$) and 499 nm ($\epsilon = 2.0 \times 10^4$ L mol⁻¹ cm⁻¹). Further comparison of the latter band with the main optical absorption of BrCPAF-C₂ (Fig. 1c) centered at λ_{max} 492 nm ($\epsilon = 1.8 \times 10^4$ L mol⁻¹ cm⁻¹) also showed a good match with a slight red-shift of absorption characteristics of DCE–diphenylaminofluorene moiety, along with an additional aryl absorption band at 316 nm ($\epsilon = 2.8 \times 10^4$ L mol⁻¹ cm⁻¹). More importantly, the structural change of $C_{60}(>>CO-Ph)$ (Fig. 1a) to $C_{60}(>>DCE-Ph)$ (Fig. 1b) does not greatly alter its absorption spectrum, indicating the close π -conjugation linkage of DCE being associated with the fluorene moiety, not with the C_{60} cage. Thus, the 503 nm band should be attributed to the transition from the HOMO level to the LUMO level of the CPAF-C_n moiety. A red-shift of 8–12 nm from that of BrCPAF-C₂ might also imply the contribution of fullerene cage, perhaps, *via* through-space electronic interactions. When the spectrum $C_{60}(>>CPAF-C_2)$ (**8**-C₂) (Fig. 1e) was superimposed onto the combined spectrum of Fig. 1c (**6**-C₂) and either Fig. 1a (**1**) or Fig. 1b (**2**) (two independent chromophores DCE–diphenylaminofluorene and fullerene cage, respectively), the close resemblance suggested either no detectable or negligible ground-state interaction between these two moieties present in the molecule of **8**-C₂ and **8**-C₉. In the long-wavelength absorption region beyond 650 nm, a very weak characteristic steady-state absorption band of the methano[60] fullerene ($C_{60}>$) moiety became visible at 690 nm only at a high concentration of 4.4×10^{-4} M.

A solvent-dependent effect on the absorption spectrum of $C_{60}(>>CPAF-C_2)$ was observed with a trend towards increasing peak intensity going from DMF, benzonitrile (PhCN), toluene (TN) and chloroform, to *o*-dichlorobenzene (*o*-DCB), as shown in the inset of Fig. 1, roughly correlated to increasing solvent power and decreasing solvent polarity. In the case of DMF as a polar solvent, a blue-shift of λ_{max} to 471 nm with reduced peak intensity ($\epsilon = 5.9 \times 10^3$ L mol⁻¹ cm⁻¹) was detected. Characteristics of steady-state fluorescence emission of **8**-C₂ and **8**-C₉ were also found to be solvent-dependent. This variation was due to photophysical events involving different degrees of either intra-molecular energy- or electron-transfer from the photoexcited CPAF-C_n moiety to the C_{60} cage in a competitive process, and the fact that the energy-transfer process is favorable in the less polar solvent. Both processes significantly reduce the intensity of ${}^1CPAF^*-C_n$ emission. Therefore, unlike the large fluorescence emission of the model compound CPAF-C₉ showing maximum intensity centered roughly at 550 nm (Fig. 2a) in toluene or 620 nm (Fig. 2b) in THF upon excitation at 486 nm, the fluorenyl fluorescence emission of $C_{60}(>>CPAF-C_2)$ and $C_{60}(>>CPAF-C_9)$ in toluene was sharply reduced in the region of 500–650 nm, as shown in Fig. 2c and d, respectively. Similarly, a large decrease in fluorescence emission was also detected in polar PhCN. This clearly indicated an efficient fluorenyl fluorescence quenching effect by the $C_{60}>$ cage in the molecular system of $C_{60}(>>CPAF-C_n)$. In toluene, the quenching effect was accompanied with weak fluorescence emissions at 700–780 nm, as shown in Fig. 2c and d. These were comparable to the fluorescence of the model compound $C_{60}(>>CO-Ph)$ **1** showing nearly identical emission peak profiles and intensity (Fig. 2e) in the same region. Since the compound **1** contains a $C_{60}>$ cage as the sole photoactive component, thus, the fluorescence peak can be correlated to the lowest excited singlet energy of ${}^1C_{60}^*(>>CPAF-C_2)$ which was estimated to be 1.75 eV in TN and CHCl₃. A recent report proposed that the quenching could be due to an electron-transfer process from either $C_{60}[>(DCE)^{-\delta}-(DPAF)^{+\delta}-C_2]$ or $C_{60}[>{}^1(CPAF)^*-C_2]$ states to the corresponding $C_{60}^-(>CPAF^+-C_2)$ state in addition to the alternative energy-transfer process from $C_{60}[>{}^1(CPAF)^*-C_2]$ to the $C_{60}>$ moiety yielding ${}^1C_{60}^*(>>CPAF-C_2)$.¹¹ Formation of the former intra-moiety partial charge-transfer state is proposed to be feasible because of the red-shift of absorption peak of CPAF-C₉ **1** to 620 nm in THF (Fig. 2b) with a more intense peak

profile than that in toluene (TN) (Fig. 2a). Significant optical absorption characteristics change of CPAF-C₉ in these two solvents was explained by the increasing tendency to form the partial charge-transfer state, (DCE) ^{-δ}-(DPAF)^{+δ}-C₂, in THF (more polar than TN) that enhances the absorption at longer wavelengths. Furthermore, in nonpolar solvents, the intensity of the long-wavelength emission band of ¹(C₆₀>)* was found to increase as the intramolecular energy-transfer processes became more favorable. This resulted in absorption at 700–740 nm that was more intense in TN and CS₂, compared with low or negligible intensity in *o*-DCB and PhCN.

In the composition and structural characterization, the molecular ion mass of C₆₀(>CPAF-C₂) **8**-C₂ was clearly detected in both negative ion desorption chemical ionization mass spectrum (DCI⁻-MS), by a group of mass peaks with a maximum peak intensity centered at *m/z* 1197 (M⁺), and in positive ion fast atom bombardment mass spectrum (FAB⁺-MS). The latter spectrum displayed sharp mass ion peaks at *m/z* 1198 (MH⁺) and 1197 (M⁺), shown in Fig. 3a. In the case of C₆₀(>CPAF-C₉) **8**-C₉, the positive ion FAB⁺ mass spectrum (Fig. 3b) showed a group of mass ion peaks at *m/z* 1395 (MH₂⁺) and 1394 (MH⁺). It was followed by a group of mass peaks centered at *m/z* 1155, 1141, 1128 and 720 (C₆₀) matching well with the fragmented mass ion of **8**-C₉ showing the loss of two 3,5,5-trimethylhexyl (C₉) subunits and the CPAF-C₉ moiety. No other mass fragmentation peaks were found in the upper mass region in the range of *m/z* 800–1190 or 800–1390 giving a relatively simple spectrum of Fig. 3a or b, respectively. This revealed high stability of the aromatic diphenylaminofluorene moiety under mass spectroscopic measurement conditions. The fragmentation pattern fits well with the bond cleavage occurring mostly at the cyclopropanyl carbon conjunction bonds bridging the fullerene cage and CPAF-C_{*n*} moieties. These data clearly provided evidence of the mass composition of fullerene–fluorene dyads C₆₀(>CPAF-C_{*n*}) as **8**-C₂ and **8**-C₉.

In addition to IR spectrum analysis, chemical conversion of a keto group to the corresponding 1,1-dicyanoethylenyl group of C₆₀(>CPAF-C₂) was also confirmed by the disappearance of key carbonyl carbon peak at δ 190 in ¹³C NMR spectrum of C₆₀(>DPAF-C₂) and a clear upfield shift of the α-proton (H_α next to 1,1-dicyanoethylene group) chemical shift to δ 5.52, appearing as a singlet peak. The shift is 0.17 ppm upfield from δ 5.69 for H_α (next to carbonyl group) of C₆₀(>DPAF-C₂), as shown in Fig. 4c and d. It was also characterized by a large downfield shift of 0.93 ppm from δ 4.59 for the chemical shift of H_α peak as a singlet in the spectrum of CPAF-C₂ (Fig. 4b). Detailed information of all aromatic protons of C₆₀(>CPAF-C₂) and the peak assignments were given by the analysis of ¹-¹ 2D COSY NMR spectrum (Fig. 5a). Assignment of H₆ proton peak was made by its most downfield chemical shift at δ 8.11 (dd, *J* = 8 Hz, *J* = 1.2 Hz) in the spectrum of Fig. 4d due to through-space close contact with the fullerene cage. Its proton coupling with the adjacent H₅ at δ 7.78 (d, *J* = 8 Hz) was then revealed in the 2D spectrum along with the long-range proton coupling with H₈ at δ 7.99 (d, *J* = 1.6 Hz). Chemical shifts of these phenyl protons at C₆, C₈ and C₅ of the fluorene ring were shifted downfield from δ 7.69 (d, *J* = 8 Hz), 7.63 (d, *J* = 1.6 Hz) and 7.62 (d, *J* = 8 Hz), respectively, in the spectrum of CPAF-C₂ (Fig. 4b) due to the influence of the fullereryl cage current. This suggested the successful cyclopropanation reaction of CPAF-C₂ (**6**-C₂) with C₆₀. The degree of downfield chemical shift is apparently less than the corresponding fluorenyl protons of C₆₀(>DPAF-C₂) (**7**-C₂, Fig. 4c) at δ 8.48 (dd, *J* = 8 Hz, *J* = 1.6 Hz), 8.32 (d, *J* = 1.6 Hz) and 7.83 (d, *J* = 8 Hz), respectively. Most importantly, large downfield chemical shifts of three fluorenyl protons located in close vicinity of C₆₀> clearly revealed strong electronic interactions between CPAF-C_{*n*} antenna and C₆₀> moieties.

According to the assignment of fluorenyl protons of the dyad C₆₀(>CPAF-C₂), chemical shifts of the corresponding carbons, C₆, C₈ and C₅, were assigned at δ 128, 123 and 120, respectively, based on the analysis of ¹-¹³C 2D HMQC NMR spectrum, as shown in Fig. 5b. In the same spectrum, chemical shifts of the cyclopropanyl or methanofullerene carbon C₆₁, alkyl carbon C₁₀ and methyl carbon of **8**-C₂ were determined to be δ 41, 33 and 8.6, respectively, with the

remaining alkyl carbon peak at δ 57 being assigned to the ring junction carbon C₉. The 2D correlation of fluorenyl carbons to their corresponding aryl protons allowed us to differentiate aromatic carbon peaks in Fig. 6 into three regions as marked with chemical shifts of three aminoaryl carbons in CPAF-C₂ moiety being assigned as δ 153, 151 and 149. Chemical shifts of the three types of functional carbons, $-C=C(CN)_2$, $-C\equiv N$ and $=C(CN)_2$, in the 1,1-dicyanoethylenyl moiety of C₆₀(>CPAF-C₂) were found to be δ 169, 113 and 88, respectively, in Fig. 6c. The assignment was made by comparison with the spectrum of one reference compound iso-propylidenemalononitrile. They also agree well with those of the model compound C₆₀(>DCE-Ph) **2** showing the corresponding carbon peaks (Fig. 6a) at δ 169, 113 and 90, respectively. A simple phenyl group of **2** makes it easy to identify its four aryl carbons in the region of δ 128–135, with the remainder of fullereryl sp² carbon peaks located within δ 136–148, that were used as the reference for the differentiation of fullerene cage carbon peaks derived from C₆₀(>DPAF-C₂) and C₆₀(>CPAF-C₂). Accordingly, the chemical shift of two fullereryl sp³ carbons, C_{F1} and C_{F2}, are close to each other as a peak at δ 73. A total of 28 peak signals in the region of 135 to 148 ppm, as marked in Fig. 6, are accounted for by the remaining 58 sp² fullereryl carbons that fits well with a C₂ molecular symmetry.

To unambiguously determine the structure of C₆₀(>CPAF-C₂), single crystals of **8**-C₂ were grown by the slow diffusion method in a H-tube using a co-solvent mixture of CS₂–ethanol–acetone. X-Ray crystallographic analysis of one single crystal effectively resolved and confirmed the molecular structure of **8**-C₂. Collection of crystallographic data and the structural refinement gave the results provided in the Experimental section (see also ESI[†]). Accordingly, a perspective 3-D ORTEP view and its molecular unit cell packing are displayed in Fig. 7a and b, respectively. The empirical formula of the single crystal was reconstituted to a composition of (C₉₄H₂₇N₃)_{1.0}(C₃H₆O)_{1.0}(C₂H₆O)_{0.5}(CS₂)_{0.25} as a mixture of (**8**-C₂)_{1.0}(acetone)_{1.0}(ethanol)_{0.5}(CS₂)_{0.25}. Interestingly, a highly ordered array of the fullerene cages along the *b* axis of the unit cell was observed with the planar DCE-diphenylaminofluorene (CPAF-C₂) rings located on the top to each other in the *a*–*c* plane of the unit cell to maximize π – π interaction between CPAF-C₂ rings (Fig. 7b). This showed the intermolecular forces involved in the molecular assembly of C₆₀(>CPAF-C₂) that were dominated by the strong fullereryl π -interactions. Two single-bond lengths connecting the methanocarbon and two fullereryl carbons in the cyclopropanyl ring, C_{F1}–C₆₁ and C_{F2}–C₆₁, were found to be 1.502 and 1.495 Å. The distance between two fullereryl carbons in the cyclopropanyl ring C_{F1}–C_{F2} is 1.614 Å, longer than the normal single C–C bond length of ~1.54 Å, that leads to the three angles of the cyclopropanyl ring as 65.2(2), 57.2(2) and 57.6(2)° with the former largest angle located at the methanocarbon. Interestingly, the closest distance between one $-C\equiv N$ group and the fullerene cage having the DCE moiety in a co-planar structure of fluorene ring was estimated to be ~2.6 Å (Fig. 7c) shorter than the estimated distance (~3.5 Å) between the $-C=O$ group and the fullerene cage by the single-crystal structural analysis of C₆₀(>DPAF-C₂) **7**-C₂.¹²

Electrochemical and time-resolved fluorescence decay measurements of C₆₀(>CPAF-C_{*n*})

Electrochemical redox potential values of C₆₀(>CPAF-C_{*n*}) were measured using cyclic voltammetry (CV) carried out in either deaerated PhCN or *o*-DCB containing tetra(*n*-butyl) ammonium perchlorate salt, (n-Bu)₄N⁺ClO₄[−], as a supporting electrolyte. The recorded electrochemical potential values were referenced to the ferrocene–ferrocenium (Fc/Fc⁺) redox

[†]Electronic supplementary information (ESI) available: Fig. S1: ¹H NMR spectrum of C₆₀(>DCE-Ph), Fig. S2: Infrared spectra of (a) C₆₀(>DPAF-C₉) and (b) C₆₀(>CPAF-C₉), Fig. S3: Infrared spectra of (a) C₆₀(>DPAF-C₂) and (b) C₆₀(>CPAF-C₂) for comparison, Fig. S4: ¹H NMR spectrum of C₆₀(>CPAF-C₂), Fig. S5: ¹³C NMR spectra of (a) C₆₀(>CPAF-C₂) and (b) expanded aromatic carbon peaks region, Fig. S6: ¹³C NMR spectra of (a) C₆₀(>DPAF-C₉) **7**-C₉ and (b) C₆₀(>CPAF-C₉) **8**-C₉. Fig. S7: Negative ion desorption chemical ionization mass spectrum (DCI[−]-MS) of C₆₀(>CPAF-C₂). CCDC reference number 762136. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0jm00037j

couple as an internal standard. Previously reported CV measurements on the sample of $C_{60}(>>DPAF-C_2)$ in deaerated PhCN (Fig. 8a)¹⁶ were used as the reference in this study for comparison. A different solvent was used to maximize the reversibility of electrochemical redoxwaves of either the $C_{60}>$ or CPAF- C_n moiety measured. In the case of $C_{60}(>>CPAF-C_2)$ in deaerated PhCN, its CV profile (Fig. 8b) showed two reversible redox waves with the first E_1^{ox} and second E_2^{ox} values of the CPAF- C_2 moiety observed at +0.58 and +0.70 V vs. Fc/Fc⁺, respectively, in close resemblance to those reported.¹¹ It was accompanied with the corresponding re-reductive waves centered at +0.63 V for E_2^{red} and ~+0.45 V for E_1^{red} . The value of E_1^{ox} is in a close range to the E_1^{ox} value (+0.62 V) of the model compound CPAF- C_2 in PhCN. In a separate measurement on the electrochemical reduction processes of **8**- C_2 in *o*-DCB (Fig. 8c), three reversible redox waves were detected with the first E_1^{red} , second E_2^{red} and third E_3^{red} values of the C_{60} cage moiety at -0.90, -1.17 and -1.40 V vs. Fc/Fc⁺, respectively, with the corresponding re-oxidative waves centered at -1.30 V for E_3^{ox} , -0.93 V for E_2^{ox} and -0.61 V for E_1^{ox} . The E_1^{red} potential value is slightly negative to that of pristine C_{60} (E_1^{red} = -0.93 vs. Fc/Fc⁺).¹⁹ In comparing the electrochemical characteristics between $C_{60}(>>DPAF-C_2)$ and $C_{60}(>>CPAF-C_2)$, we observed that changing the keto moiety to a DCE group increases the electron uptake capability of the fullerene cage in **8**- C_2 , based on the detected first and second reduction potentials changing from -1.03 and -1.45 V (Fig. 8a), respectively, to -0.90 and -1.17 V (Fig. 8c), respectively. It also decreases the electron donating ability of diphenylaminofluorene moiety of **8**- C_2 with an increase of the E_1^{ox} value from +0.48 V for **7**- C_2 to +0.58 V. Since the redox potentials in covalent-bound donor-acceptor systems are essential for the evaluation of these optoelectronic materials in the photocell applications, the overall increase of molecular electronegativity of **8**- C_2 may alter the photoinduced transient electron-transfer characteristics and the decay time of the charge-separated transient states from those of $C_{60}(>>DPAF-C_2)$ in a solvent-dependent manner.

Photophysical properties of the C_{60} -DCE-DPAF assemblies involve the primary photoexcitation events of either the fullerene moiety at UV wavelengths or the DPAF- C_n moiety at both UV and visible wavelengths up to 600 nm (Fig. 1). Much higher optical absorption capability of DPAF- C_n than the $C_{60}>$ cage in visible wavelengths enables the former moiety to serve as a light-harvesting antenna. Accordingly, formation of the photoexcited ¹(DPAF)*- C_2 moiety should be considered as the early event in the photophysical process. Alteration of the keto group of **7**- C_2 to the 1,1-dicyanoethylenyl group of $C_{60}(>>CPAF-C_2)$ effectively extended its photoresponsive region to longer red wavelengths. Photoexcitation processes of **7**- C_n and **8**- C_n pump an electron from their highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). In the case of the similar structurally analogous $C_{60}(>>CPAF-C_{2M})$ **8**- C_{2M} in the molecular orbital calculation and energy minimization, the majority of the HOMO electron density was reported to be delocalized over the diphenylaminofluorene (DPAF) moiety, whereas the LUMO electron density was located on the C_{60} spheroid, and therefore $C_{60}^-(>CPAF^+-C_{2M})$ was suggested as the most stable charge-separated (CS) state.¹¹ The same argument can be applied for the formation of $C_{60}^-(>CPAF^+-C_2)$ and $C_{60}^-(>CPAF^+-C_9)$ states owing to close similarity of the core structure. These charge-separated states may be generated by photoinduced intramolecular electron-transfer between the diphenylaminofluorene donor and $C_{60}>$ acceptor moieties. The process effectively quenches fluorenyl fluorescence that can be observed in the most of **7**- C_n and **8**- C_n monoadducts.

By using the same arguments reported for **8**- C_{2M} ,¹¹ other plausible fluorescence quenching pathways of photoexcited $C_{60}[>^1(CPAF)^*-C_2]$ may involve ultrafast intramolecular energy-transfer processes going from ¹(CPAF)*- C_2 to the $C_{60}>$ moiety yielding the excited ¹ $C_{60}^+(>CPAF-C_2)$ state that resulted in fluorenyl fluorescence suppression. The energy-transfer route was observed predominantly in nonpolar solvents as reported previously.¹⁶ It was indicated by and associated with a weak ¹($C_{60}>$)*-derived fullereryl fluorescence. The

intensity of this emission band at 710 nm was found to be highly sensitive to the solvent polarity. Variation of the solvent polarity from that of TN and CS₂ increases the chance of a competitive electron-transfer process occurring on the same compound. Identification of photoinduced transient states can be made by performing time-resolved picosecond fluorescence emission measurements with photoexcitation mainly at the DPAF-C_n or CPAF-C_n moiety. It is immediately followed by the detection of transient fluorescence bands at either 600 or 700 nm at a different short-time scale for the indication of C₆₀[¹(CPAF)*-C₂] or ¹C₆₀*(>CPAF-C₂) transient state formation, respectively. Progressive fluorescence intensity decrease and decay-time profiles of these two bands are then monitored for understanding the quenching mechanism and following the kinetics of photo-induced processes. By using the C₆₀(>CPAF-C_{2M}) sample in TN as an example, the transient lifetime of the ¹(CPAF)*-C_{2M} moiety was evaluated based on the decay-time profile of the band at 600 nm and reported to be ~53 ps.¹¹ A short decay time reveals efficient fluorescence quenching by the C₆₀> cage yielding the corresponding excited ¹C₆₀*(>CPAF-C_{2M}) state. That allowed the detection of a new emission band of the ¹(C₆₀>)* at 715–720 nm appearing immediately after considerable quenching of the C₆₀[¹(CPAF)*-C_{2M}] state in nonpolar TN and CS₂.

When the fluorescence emission of ¹(C₆₀>)* in TN reached the steady state, the lifetimes (τ_s) of ¹C₆₀*(>CPAF-C₂) and ¹C₆₀*(>CPAF-C₉) were evaluated to be 1521 and 1510 ps, respectively, as shown in Table 1. These values were found to be in a similar range as those of C₆₀(>DPAF-C₉) (1650 ps)¹⁶ and C₆₀.²⁰ They are slightly longer than the lifetime of ¹C₆₀*(>CO-Ph) (1350 ps) measured at 708 nm. It is also interesting to note that, unlike the τ_s value of the model keto-compound Br-¹DPAF*-C₉ (2125 ps), the fluorescence lifetime of the model DCE-compound ¹CPAF*-C₉ is rather short (241 ps). This may be indicative of a facile photoinduced intramolecular charge polarization process forming the corresponding (DCE)⁻-DPAF⁺-C₉ charge-separated state, consistent with the recently proposed photoinduced partial inter-moiety electron-transfer (DCE)^{-δ}-DPAF^{+δ}-C_{2M} state.¹¹

Nanosecond transient spectroscopic measurements of C₆₀(>CPAF-C_n)

Confirmation of the generation of either charge-separated states or fullereryl triplet transient states of C₆₀(>CPAF-C_n) was made by the measurement of nanosecond transient absorption spectra, followed by monitoring their charge-recombination processes or triplet energy transfer, respectively, in TN. Prior to the study, we determined the optical absorption wavelength (λ_{max}) of the cation radical CPAF⁺-C₂ reference species by the detection of a new near-IR band appearing at 875 nm in the spectrum upon the treatment of the model compound CPAF-C₂ with a strong oxidizing agent FeCl₃ in solution. In the presence of the charge-transfer mode of C₆₀(>CPAF-C₂), this band should accompanied by an additional broad absorption band of (C₆₀>)⁻ radical-ion pairs centered at 1020 nm. Therefore, by the detection of these transient absorption bands, the formation of photoinduced charge-separated transient state C₆₀⁻(>CPAF⁺-C₂) can be confirmed. In other word, the appearance of a new broad transient band centered at ~700–740 nm, corresponding to the T₁-T_n absorption of ³(C₆₀>)*, should reveal clearly the formation of ³C₆₀*(>CPAF-C_n) in the photoprocesses that can be correlated to the occurrence of intramolecular energy-transfer events.

In the current transient experiments, laser flash photolysis of the samples was carried out by irradiation at 486 nm (λ_{ex}). All samples were prepared in the same O.D. of 0.2 at the excitation wavelength to allow direct comparison of the triplet excited state formation with the indication of a relative quantity. A model compound C₆₀(>CO-Ph) **1** was used for the correlation of spectral profiles of all C₆₀-antenna samples. Upon photoexcitation of **1** in TN at 486 nm in the nanosecond region, the detected transient absorption spectral profile (Fig. 9a) showed a broad peak centered at 710 nm, which is attributed to the absorption of the ³(C₆₀>)* moiety since the C₆₀> cage is the only chromophore moiety in **1**. Interestingly, the same photoexcitation process

carried out on samples of $C_{60}(>>CPAF-C_9)$ **8-C₉** and $C_{60}(>>CPAF-C_2)$ **8-C₂** in TN also displayed a similar transient absorption spectral profile with a major peak centered at 720 nm, as shown in Fig. 9b and c, respectively. The lack of two characteristic bands arising from moieties of CPAF⁺-C_n and (C₆₀>)⁻ radical-ion pairs clearly ruled out the possible formation of charge-separated C₆₀⁻(>>CPAF⁺-C₂) and C₆₀⁻(>>CPAF⁺-C₉) states. Apparently, photoexcitation performed on the fluorenyl CPAF-C_n ring moiety of **8-C_n** induced the formation of ³C₆₀*(>CPAF-C_n) transient state during the subsequent photo-processes. It is worthwhile to note that the quantity of triplet excited state formed for C₆₀(>>CO-Ph) was more than the other two C₆₀(>>CPAF-C_n) systems, indicating non-quantitative occurrence of the energy-transfer process in the latter two materials. This is consistent with the steady-state fluorescence data. Evidently, the reduced peak intensity at 720 nm of Fig. 9b and c from that of Fig. 9a represents the corresponding similar extent of C₆₀⁻(>>CPAF⁺-C_n) formation if there is no energy loss during the photoevents. Finally, the lifetime of ³C₆₀*(>CPAF-C₂) and ³C₆₀*(>CPAF-C₉) transient states was found to be 34 and 39 μs, respectively, as shown in Table 1, in a similar range as that of ³C₆₀*(>DPAF-C₉) (35 μs). These values are longer than that (24 μs) of ³C₆₀*(>CO-Ph) as measured by the intensity decay of the 720 nm band.

Efficient singlet oxygen generation of C₆₀(>>CPAF-C₂) in toluene

Predominant generation of ³C₆₀*(>CPAF-C_n) as detected by laser excitation of C₆₀(>>CPAF-C_n) in TN prompted us to investigate the photosensitized production of singlet oxygen (¹O₂) in the presence of molecular oxygen. Formation of ¹O₂ becomes possible *via* intermolecular triplet energy transfer from ³C₆₀*(>CPAF-C_n) to O₂. Our recent examples of the efficient singlet oxygen production in biological medium using hydrophilic molecular micelle-like C₆₀ derivatives (FC₄S), in the form of nanosphere structures,^{9b} as photosensitizers allowed the demonstration of cytotoxicity after single-photon excitation based PDT (1γ-PDT) against cancer cells *in vitro* and tumors *in vivo*.^{9a} Direct detection of ¹O₂ was accomplished by observing its fluorescence emission at 1270 nm during the quenching process. The production of ¹O₂ is also accompanied with a considerable increase in the decay rate of ³C₆₀*(>CPAF-C₂) at 710–740 nm in the presence of O₂. As a result, a significantly strong fluorescence emission of singlet oxygen upon photoexcitation of C₆₀(>>CPAF-C₂) and C₆₀(>>CPAF-C_{2M}) in TN suggested its effective quenching of the ³(C₆₀>)* moiety in a ¹O₂ production yield close to that of pristine C₆₀.¹¹ Synthesis of C₆₀(>>CPAF-C_{2M}) was made by the malononitration of **7-C_{2M}** in the presence of titanium tetrachloride and pyridine, as shown in Scheme 1.

By the application of time-resolved fluorescence emission measurement of ¹O₂ at 1270 nm upon photoexcitation using a laser light source at 523 nm (λ_{ex}) with a power of ~10.4 mW, we were able to compare the relative singlet oxygen production efficiency between C₆₀(>>CPAF-C_n) and its precursor C₆₀(>>DPAF-C_n). The intensity varied as a function of the concentration, the number of laser pulses, and the laser intensity. As an example, we recorded the emission intensity of ¹O₂ fluorescence arising from C₆₀(>>CPAF-C_{2M}) **8-C_{2M}** (Fig. 10a) and C₆₀(>>DPAF-C_{2M}) **7-C_{2M}** (Fig. 10b) to be 3.0 × 10⁵ and 5.0 × 10⁴ M⁻¹ cm⁻¹, respectively, at a concentration of 1.0 × 10⁻⁵ M in TN. The latter value of C₆₀(>>DPAF-C_{2M}) was found to be comparable to the value found for tetraphenylporphyrin used as a photosensitizer (Fig. 10c). Remarkably, the ¹O₂ production yield of C₆₀(>>CPAF-C_{2M}) was found to be nearly 6-fold higher than its keto analogous **7-C_{2M}**, indicating the large light-harvesting enhancement by the CPAF-C_{2M} moiety and subsequent efficient triplet state generation of the C₆₀> cage moiety. The lifetimes of ¹O₂ generated from C₆₀(>>CPAF-C_{2M}), C₆₀(>>DPAF-C_{2M}) and tetraphenylporphyrin in TN were estimated to be 32, 31 and 31 μs, respectively. The observation revealed the population mechanism of the ¹C₆₀*(>CPAF-C_{2M}) state arising from the energy-transfer process since the direct photoexcitation of the C₆₀> moiety to ¹(C₆₀>)* is unlikely to occur with the 523 nm light source. Furthermore, the fluorescence intensity of ¹C₆₀*(>DPAF-C_{2M}) and ¹C₆₀*(>CPAF-C_{2M}) was significantly quenched by increasing the

solvent polarity from TN to PhCN and DMF that led to a nearly disappearance of $^1\text{O}_2$ luminescence in DMF (inset of Fig. 10). The observation revealed the domination of the $\text{C}_{60}^-(\text{>DPAF}^+-\text{C}_{2M})$ charge-separated state in polar solvents, such as PhCN and DMF.

Photodynamic therapy killing of human cancer cells

We tested $\text{C}_{60}(\text{>DPAF}-\text{C}_{2M})$ (**7**- C_{2M}) and $\text{C}_{60}(\text{>CPAF}-\text{C}_{2M})$ (**8**- C_{2M}) as photosensitizers to kill cancer cells after illumination. We used the human cervical cancer cell line, HeLa and incubated the cells for 3.0 h with increasing concentrations of **7**- C_{2M} and **8**- C_{2M} dissolved in DMA. Broad-band white light irradiation (200 J cm^{-2}) was delivered and the cells were returned to the incubator overnight. A 4-h MTT assay was then carried out for mitochondrial reductase activity as a surrogate measure of the cell viability.

Comparison of survival fractions for HeLa cells treated with **7**- C_{2M} and **8**- C_{2M} (1.0–5.0 μM) by either keeping in the dark or illuminated with 200 J cm^{-2} white light is depicted in Fig. 11. A significant, dose-dependent loss of viability in the presence of light was observed using **8**- C_{2M} as the photosensitizer. The phenomena were not seen either with **8**- C_{2M} in the dark or with **7**- C_{2M} in either light or dark conditions. These data clearly show the markedly enhanced photocatalytic activity of $\text{C}_{60}(\text{>CPAF}-\text{C}_{2M})$ compared to that of $\text{C}_{60}(\text{>DPAF}-\text{C}_{2M})$, due to its higher absorption intensity in visible wavelengths and an increased singlet oxygen quantum yield of the former compound.

Conclusion

A new class of photoresponsive C_{60} -DCE-diphenylaminofluorene (DPAF) nanostructures, $\text{C}_{60}(\text{>CPAF}-\text{C}_n)$, was synthesized and characterized. Structural modification was made by chemical conversion of the keto group in $\text{C}_{60}(\text{>DPAF}-\text{C}_n)$ to a strong electron-withdrawing DCE subunit that resulted in significant increase of electronic polarization in the resulting CPAF- C_n moiety. The modification also produces a large bathochromic shift of the main optical absorption band in the visible spectrum up to 600 nm that extends the photoresponse capability of C_{60} -DCE-DPAF nanostructures to longer red wavelengths than $\text{C}_{60}(\text{>DPAF}-\text{C}_n)$. Accordingly, $\text{C}_{60}(\text{>CPAF}-\text{C}_n)$ should allow the operation of 2γ -PDT at the light wavelength of 1000–1200 nm for the enhanced tissue penetration depth.

Based on the evaluation of fluorescence emission and nanosecond transient absorption data in toluene, we proposed a rapid quenching pathway of the photoexcited $\text{C}_{60}[\text{>}^1(\text{CPAF})^*-\text{C}_n]$ transient state leading to a high population of $^1\text{C}_{60}^*(\text{>CPAF}-\text{C}_n)$ state and subsequently the formation of $^3\text{C}_{60}^*(\text{>CPAF}-\text{C}_n)$ transient state *via* intersystem-crossing processes. The latter process leads to an efficient production of singlet oxygen by $\text{C}_{60}(\text{>DPAF}-\text{C}_{2M})$, which is comparable to that of tetraphenylporphyrin. Remarkably, the $^1\text{O}_2$ quantum yield of $\text{C}_{60}(\text{>CPAF}-\text{C}_{2M})$ was found to be nearly 6-fold higher than that of its keto analogous $\text{C}_{60}(\text{>DPAF}-\text{C}_{2M})$, demonstrating the large light-harvesting enhancement of the CPAF- C_{2M} moiety and subsequent efficient triplet state generation of the C_{60} > cage moiety. We also demonstrated the feasibility of efficient photosensitizing generation of cytotoxic $^1\text{O}_2$ *via* intermolecular triplet energy transfer of $^3\text{C}_{60}^*(\text{>CPAF}-\text{C}_n)$ state with molecular oxygen, as a crucial mechanism in the field of photodynamic therapy. This led to highly effective killing of HeLa cells by $\text{C}_{60}(\text{>CPAF}-\text{C}_{2M})$ *via* 1γ -PDT. Finally, $\text{C}_{60}(\text{>CPAF}-\text{C}_n)$ exhibits large two-photon absorption cross-sections at both 780 and 1000 nm enabling its potential uses as the 2γ -PDT photosensitizer in the biological window of 800–1100 nm. This serves as a significant advantage over tetraphenylporphyrin-related PDT agents for 2γ -PDT applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. UV-vis spectra of (a) C₆₀(>CO-Ph) **1**, (b) C₆₀(>DCE-Ph) **2**, (c) BrCPAF-C₂ **6**-C₂, (d) C₆₀(>DPAF-C₂) **7**-C₂ and (e) C₆₀(>CPAF-C₂) **8**-C₂ in CHCl₃ at 2.0 × 10⁻⁵ M and (a') the absorption peak of the C₆₀ moiety taken at 4.4 × 10⁻⁴ M. The inset shows the solvent-dependent intensity of the 490–505 nm band.

Fig. 2. Steady-state fluorescence spectra of the model compound CPAF-C₉ in (a) TN and (b) THF; fluorescence spectra of (c) C₆₀(>CPAF-C₂) **8**-C₂, (d) C₆₀(>CPAF-C₉) **8**-C₉ and (e) the reference C₆₀(>CO-Ph) **1** in TN with the excitation wavelength (λ_{ex}) of 486 nm.

Fig. 3. Positive ion fast atom bombardment (FAB⁺) mass spectra of (a) C₆₀(>CPAF-C₂) **8**-C₂ and (b) C₆₀(>CPAF-C₉) **8**-C₉.

Fig. 4.
¹H NMR spectra (CDCl₃) of (a) DPAF-C₂, (b) CPAF-C₂, (c) C₆₀(>DPAF-C₂) and (d) C₆₀(>CPAF-C₂) showing downfield shift of fluorenyl proton peaks adjacent to the C₆₀ cage.

Fig. 5.
(a) ^1H 2D COSY NMR and (b) ^{13}C 2D HMQC NMR spectra (CDCl_3) of $\text{C}_{60}(>\text{CPAF-C}_2)_8\text{-C}_2$.

Fig. 6.
 ^{13}C NMR spectra of (a) $\text{C}_{60}(>\text{DPAF-C}_9)$ **7**- C_9 and (b) $\text{C}_{60}(>\text{CPAF-C}_9)$ **8**- C_9 .

Fig. 7. (a) Perspective ORTEP view and (b) the unit molecular packing of $C_{60}(>CPAF-C_2)$ in the crystal with solvent molecules and hydrogen atoms omitted for clarity and (c) a 3-D model of **7**-C₂ or **8**-C₂ to show the closest estimated distance between C=O or C≡N group, respectively, and the fullerene cage.

Fig. 8.

Cyclic voltammetry of (a) $\text{C}_{60}(>\text{DPAF}-\text{C}_2)$ in PhCN and $\text{C}_{60}(>\text{CPAF}-\text{C}_2)$ ($1.0 \times 10^{-4} \text{ M}$) in (b) PhCN showing redox waves of the CPAF- C_2 moiety and (c) *o*-DCB showing redox waves of the C_{60} cage moiety.

Fig. 9.

Transient absorption spectra obtained by flash laser photolysis of (a) the model compound C₆₀(>CO-Ph) **1**, (b) C₆₀(>CPAF-C₉) **8**-C₉ and (c) C₆₀(>CPAF-C₂) **8**-C₂ at the excitation wavelength (λ_{ex}) of 486 nm in toluene.

Fig. 10. Time-resolved fluorescence emission and decay of $^1\text{O}_2$ upon photoexcitation of (a) $\text{C}_{60}(>\text{CPAF-C}_{2\text{M}})$ and (b) $\text{C}_{60}(>\text{DPAF-C}_{2\text{M}})$ in comparison with that of (c) tetraphenylporphyrin in TN at a concentration of $1.0 \times 10^{-5} \text{ M}$ with the laser intensity of $\sim 10.4 \text{ mW}$. Inset peak profiles show the solvent-dependent emission intensity of $\text{C}_{60}(>\text{DPAF-C}_{2\text{M}})$.

Fig. 11. Concentration and light-dependent killing of human cancer cells (HeLa) by $\text{C}_{60}(>\text{CPAF-C}_{2\text{M}})$ and $\text{C}_{60}(>\text{DPAF-C}_{2\text{M}})$. The cells were incubated for 3.0 h with specified concentrations of the fullerene derivative diluted from DMA into complete medium and then treated with or without a broad-band white light. The next day viability was assayed with a 4-h MTT assay.

Scheme 1.

Synthetic route for the preparation of C₆₀(>CPAF-C₂) and C₆₀(>CPAF-C₉). *Reagents and conditions:* i, α -bromoacetyl bromide, AlCl₃, ClCH₂CH₂Cl, 0 °C to rt, 4.0 h; ii, C₆₀, DBU, toluene, rt, 5.0 h; iii, malononitrile, pyridine, TiCl₄, rt, 5.0 min.

Table 1

Photophysical properties of $C_{60}(>CPAF-C_n)$ and related model compounds in toluene, including the lifetimes τ_s of transient ${}^1C_{60}^*(>CPAF-C_n)$ state and τ_t of transient ${}^3C_{60}^*(>CPAF-C_n)$ state

Compound	$\lambda_{max}(Abs)/nm$	$\epsilon/M^{-1} cm^{-1}$	$\lambda_{max}(FL)/nm$	$\tau_d({}^1(C_{60})^*)/ps$	$\lambda_{max}(T_1-T_n, Abs)/nm$	$\tau_t({}^3(C_{60})^*)/\mu s$
$C_{60}(>CPAF-C_2)$	487	22500 ± 1700	715	1521	720	34
$C_{60}(>CPAF-C_9)$	487	23500 ± 300	717	1510	720	39
CPAF- C_9	429	12900 ± 600	546	241	—	—
$C_{60}(>DPAF-C_9)$	405	40400 ± 200	708	1650	710	35
$C_{60}(>CO-Ph)$	332	48400 ± 200	708	1350	720	24
BrDPAF- C_9	395	24600 ± 300	439	2125	600	305

^aDeoxygenated by three freeze-pump-thaw cycles.