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C_{60}-containing nanostructured polymeric materials with potential biomedical applications

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Abstract

Star-shaped polymers with a fullerene (C_{60}) core and an unexpanded structure were successfully prepared by reaction of C_{60} with amino end-capped polyesters $H_xC_{60}(NHPCL_n)_x$ and polyethers $H_xC_{60}(NHPEG_n)_x$, respectively. Upon irradiation of these C_{60}-derivatives, a large amount of singlet oxygen was released. Compared to previously synthesized star-shaped azafulleroids with an expanded structure, the photosensitivity of $H_xC_{60}(NHPCL_n)_x$ is higher as assessed by a higher production of singlet oxygen. The cytotoxicity of the photoactive water-soluble $H_xC_{60}(NHPEG_n)_x$ derivatives was tested against THP-1 cells and expressed in terms of cell viability. Moreover, they were processed as micro-/nanosized fibers by electrospinning, which however required the addition of poly(ε-caprolactone) (PCL). The diameter distribution of the fibers was trimodal, where the fraction with the 270 nm average diameter was the major population. Because of their photoactivity, the herein reported star-shaped C_{60}-derivatives are promising candidates for photodynamic cancer therapy and treatment of multidrug resistant pathogens.

Keywords: Fullerene; Electrospinning; Fullerene-core star-shaped polymers

1. Introduction

The photosensitivity of fullerene (C_{60}) makes it a good candidate for the photodynamic therapy of cancers [1]. However, lack of solubility and processability is a severe limitation to the application of C_{60} to biological systems [2]. It is thus highly desirable to combine C_{60} with polymers selected for their water-solubility, biodegradability and biocompatibility. In this respect, the propensity of fullerene for adding various reagents with formation of carbon–carbon and carbon–heteroatom bonds could be exploited. Addition may take place across either the 6,6-ring junction or the 5,6-ring junction, and the rings are either open (expanded) fulleroids or closed (unexpanded) fullerene structures, which results in the four possible configurations [3], as shown in Scheme 1.

The addition of alkyl azides to C_{60} was thoroughly investigated. It proceeds via intermediate triazolines, which after elimination of nitrogen rearrange mainly to open 1,6-aza-bridged isomers together with minor amount of closed 1,2-aza-bridged isomers [4]. Therefore, the reaction of azides takes place across a [5,6]-ring junction and gives rise to a fulleroid and thus to the expansion of the fullerene cage [3]. In contrast, the amine addition takes place across the [6,6]-ring junction with formation of a closed, unexpanded structure [5]. Moreover, it was shown that the addition of monofunctional linear or cyclic aminopolyethers is a simple method for imparting solubility and even water-solubility to the intrinsically hydrophobic C_{60} [6]. These conjugates were injected into living tissues followed by light irradiation. A histological

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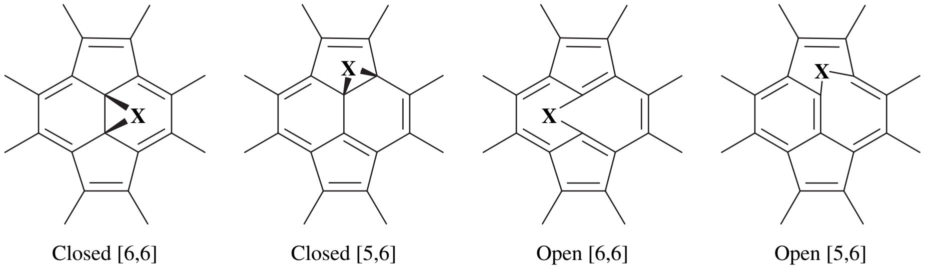


Scheme 1. Possible configurations of [60]fullerene.

examination emphasized the necrosis of tumors without damage of the surrounding normal tissues [7]. Moreover, the antitumor action of the conjugates increases with the irradiation power and the C_{60} dose.

Recently, we reported on the synthesis of star-shaped poly(ε-caprolactone) (PCL) with a fullerene core by reaction of azide-terminated PCL with C_{60} [8]. The accordingly formed C_{60}-nanohybrids with an expanded structure were processed into nanoparticles, whereas C_{60}-containing polyester fibers were prepared by electrospinning. The ability of these azafulleroids to generate significant amounts of singlet oxygen upon photoactivation makes them good candidates for the photodynamic cancer therapy.

This paper aims at synthesizing unexpanded counterparts by grafting preformed amino end-capped polyesters and polyethers onto C_{60} in order to compare their properties with those of the expanded star-shaped azafulleroids. A special emphasis was placed on the photoactivity of these C_{60}-derivatives and the amount of the generated singlet oxygen. The cytotoxicity of the water-soluble C_{60}-polyether derivatives was tested towards THP-1 cells. Finally, these derivatives were electrospun as fibers, that could be used in photosensitive wound-healing and non-traumatic wound dressing devices.

2. Experimental

2.1. Materials

Buckminsterfullerene (98%), 12-bromo-1-dodecanol (99%), triethylaluminum (1.9 M in toluene), sodium azide (99%), palladium on activated carbon (10 wt%) and ammonium formate were purchased from Aldrich and used as received. Commercially available *O*-(2-aminoethyl)-*O*′-(2-hydroxyethyl)poly(ethylene glycol) (H_2NPEG) with a molar mass of 2000 was supplied by Shearwater Polymers, Inc. ε-Caprolactone (Aldrich) was dried over calcium hydride for 24 h, degassed by several freeze–thawing cycles before being distilled under reduced pressure. Toluene was dried by refluxing over a sodium–benzophenone complex and distilled under nitrogen. Dimethylformamide (DMF) was dried and distilled over P_2O_5. All polymerization experiments were performed by the classical Schlenck technique under nitrogen. Liquids were transferred under nitrogen with syringes and stainless steel capillaries.

2.2. Synthesis of amino-terminated PCL

α-Hydroxy-ω-amino PCLs (H_2NPCL) were synthesized with an initiator that contained a precursor of the primary amine, as reported elsewhere [9]. Briefly, ethylaluminum 12-bromo-1-dodecyl oxide was first prepared. The bromo end-group was then converted into an azide by reaction with a 5-fold molar excess of sodium azide in dry DMF at 35 °C for 24 h. The azide end-group ([PCL] = 10 wt/v%) was hydrogenated in dry DMF by reaction with a 5-fold molar excess of ammonium formate and 10 wt% Pd supported on activated carbon, at 30 °C for 4 h. The Pd/C residues were recovered by centrifugation (20 000 rpm, 30 min). The α-hydroxy-ω-amino PCL was isolated by selective precipitation in methanol.

2.3. Grafting of amino-terminated PCL onto C_{60}

This reaction was carried out according to a method reported for monoaminopolyethers [6]. In a typical experiment, C_{60} was reacted with H_2NPCL (molar ratio = 1:10), either under heterogeneous conditions in tetrahydrofuran (THF) or under homogenous conditions in 1,2,4-trichlorobenzene (TCHB), at room temperature in the dark under stirring and nitrogen for 7 days. At the end of the heterogeneous reaction, the precipitate was removed by filtration through a 0.45 μm membrane filter and centrifugation (20 000 rpm, 30 min). Yield: 68%. In the case of homogeneous reaction, TCHB was eliminated under reduced pressure, and the solid residue was dissolved in THF. The THF solution was concentrated under reduced pressure, before being poured into an excess of methanol in order to precipitate the C_{60}-derivative $H_xC_{60}(NHPCL)_x$. Yield: 69%.

2.4. Grafting of amino-terminated PEG onto C_{60}

The reaction of amino-terminated polyether, H_2NPEG, with fullerene C_{60} was carried out according to a similar procedure [6]. C_{60} was reacted with a 10-fold molar excess of monoaminopolyether in THF (heterogeneous) or toluene (homogenous), at room temperature in the dark under stirring and nitrogen for 7 days. At the end of the heterogeneous reaction the residue, if any, was eliminated by centrifugation (20 000 rpm, 30 min), and the expected product, $H_xC_{60}(NHPEG)_x$, was precipitated into cold diethyl ether. Yield: 83%. In the case of homogeneous reaction, the solvent was removed under reduced pressure, the solid residue was dissolved in THF, the solution was centrifuged, then concentrated under reduced pressure and finally poured into an excess of cold diethyl ether for precipitating the C_{60}-derivative $H_xC_{60}(NHPEG)_x$. Yield: 63%.

2.5. Determination of singlet oxygen ($^1O_2$) generation

The 9,10-anthracene dipropionic acid (ADPA) bleaching method [10] was used to confirm that singlet oxygen was generated by the C_{60}-derivatives. For this purpose, solutions of the C_{60}-derivatives (photosensitizers) were prepared – in THF for $H_xC_{60}(NHPCL)_x$, and in water or deuterium oxide for $H_xC_{60}(NHPEG)_x$. An ADPA solution in a buffer (pH = 7.5) was used as the singlet oxygen acceptor. Mixed solutions of the photosensitizers, $H_xC_{60}(NHPCL)_x$ or $H_xC_{60}(NHPEG)_x$, and ADPA were irradiated at a well-defined wavelength (red filter), such that this radiation was selectively absorbed by the photosensitizer. The concentration of $H_xC_{60}(NHPCL)_x$ and $H_xC_{60}(NHPEG)_x$ in the mixed solutions was 0.09% and 0.13%, respectively. The reaction progress was monitored by recording the decrease of the 400 nm absorption peak of ADPA with the irradiation time.

2.6. Cell viability assay

Human promonocytic THP-1 cells (American Type Culture Collection, Rockville, MD) were cultured as previously reported [11] and maintained at 37 °C with 5% CO_2 balanced with air before use. Cytotoxicity of the water-soluble $H_xC_{60}(NHPEG)_x$ was assessed by cell viability (number of living cells found after an overnight incubation) compared to untreated cells, by using Trypan Blue as a staining agent. The stock solution of Trypan Blue (Sigma, Germany) was 1% in a phosphate buffer (PBS) at pH = 7.4. Cells were cultured in 24-well plates (5×10^5 cell/well) and then incubated overnight with $H_xC_{60}(NHPEG)_x$ at two final concentrations of 10^{-5} and 10^{-4} M, respectively. Dye solution (5 μl) was added to the cell suspension (45 μl) and then incubated for 3 min. Then 10 μl of the suspension were analyzed by light microscopy.

2.7. Electrospinning of water-soluble C_{60}-polyether derivatives

The preparation technique of C_{60}-containing micro-/nanosized fibers by electrospinning is reported elsewhere [8]. Actually, solutions of the C_{60}-polyether derivative added with a known amount of PCL ($\overline{M}_n = 80\,000$) in chloroform were electrospun. The total polymer concentration was 12 wt%. In a typical run, a syringe with a conical nozzle in which the electrode was immersed, was filled with the $H_xC_{60}(NHPEG)_x$/PCL mixed solution. The electrode was connected to a high voltage power supply that generated a positive DC voltage up to 30 kV. A grounded copper plate was used as a collector.

2.8. Characterization

Size-exclusion chromatography (SEC) was performed with (i) an SFD S5200 liquid chromatograph equipped with a dual detection, i.e. with an RI 2000 refractive index and an S3240 UV–vis detector and (ii) with a Waters liquid chromatograph with a dual detection consisting of a differential refractometer Waters 410 and a tunable absorbance detector Waters 486. Sets of PL-gel 5 μm columns (10^5, 10^4, 10^3 and 10^2 Å) calibrated with polystyrene or PEO standards were used with THF as an eluent. The flow rate was 1 ml/min at 45 °C. IR spectra were recorded with a Perkin–Elmer FT-IR spectrophotometer and KBr discs. ^1H and ^{13}C NMR spectra were recorded with a Bruker AM 400 MHz apparatus in $CDCl_3$ or D_2O at 25 °C. UV–vis absorption spectra were recorded with a Hitachi U-3300 spectrophotometer. Thermogravimetric analysis (TGA) was carried out under nitrogen with a TA Instruments Q500 thermogravimetric analyzer in the 25–1000 °C range, at a 10 °C/min rate (HiRes method). The collected micro-/nanofibers were vacuum-coated with carbon and examined with a Philips 515 scanning electron microscope. The average diameter of the fibers was estimated with an Image J software program with 20 fibers per SEM image.

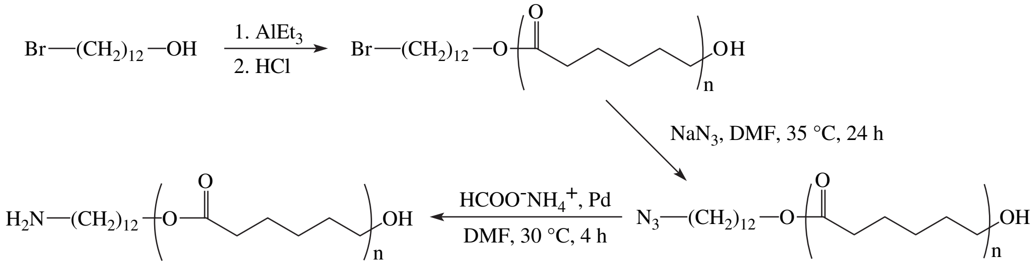
3. Results and discussion

3.1. Synthesis of C_{60}-containing star-shaped polyester, $H_xC_{60}(NHPCL)_x$

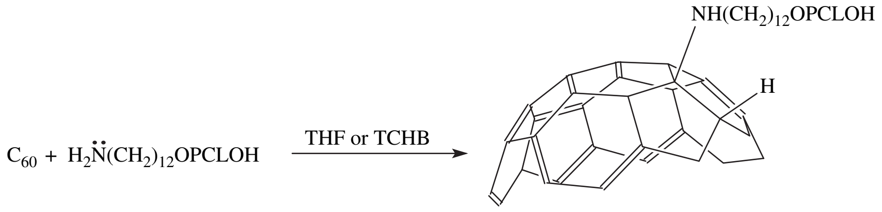
Recently, we reported on the synthesis of star-shaped PCLs with a fullerene core by reaction of azide-terminated PCLs (N_3PCL) with C_{60} [8]. The reaction of azide-containing polymers with C_{60} is known to lead to a [5,6]-open structure and thus to the expansion of the fullerene cage [12]. In contrast, the addition of amino end-capped polymers results in a closed unexpanded structure [5]. With the purpose to prepare unexpanded structures, H_2NPCLs were reacted with C_{60}. Amino-terminated PCLs of different molar masses ($\overline{M}_n = 3700$ and 11 500) were synthesized by ring-opening polymerization of ε-CL initiated by triethylaluminum in the presence of 12-bromo-1-dodecanol, followed by the quantitative conversion of the α-bromide end-group into an azide, that was finally reduced into a primary amine as illustrated in Scheme 2. ^1H NMR unambiguously showed that a primary amine was attached to the PCL as an end-group [$\delta(-CH_2NH_2) = 2.64$ ppm] and that the azide disappeared completely ($-CH_2N_3$; 3.47 ppm). Moreover, the IR absorption at 2095 cm^{-1}, which is characteristic of the azide, also disappeared.

C_{60}–aminopolyester, $H_xC_{60}(NHPCL_n)_x$, was prepared by reaction of C_{60} with a 10-fold molar excess of H_2NPCL in THF (heterogeneous) or TCHB (homogenous). Most probably, C_{60} reacts with H_2NPCL according to the mechanism proposed for the reaction of C_{60} with amino polymers [5], thus with the formation of [5,6]-closed structures, as illustrated in Scheme 3.

Under the heterogeneous conditions in THF, the colourless H_2NPCL solution turned brown consistent with the solubility



Scheme 2. Synthesis of amino-terminated PCL.



Scheme 3. Coupling of amino-terminated PCL to C_{60}.

of C_{60} in THF as a result of interaction with H_2NPCL. The violet colour of the C_{60} solution in TCHB turned red-brown upon reaction with H_2NPCL. The reaction products were solid and soluble in THF in contrast to untreated C_{60}.

The maximum UV–vis absorption of C_{60} decreased during reaction and finally disappeared. The $H_xC_{60}(NHPCL)_x$ reaction product absorbed in the 300–700 nm region although no pronounced maximum was observed. Similar changes were reported for other C_{60}–polymer conjugates [13] in line with a change in the C_{60} structure upon grafting onto amino-terminated polymers.

The absorption of C_{60} in the 340 nm region was convenient to detect polyester-modified fullerenes by SEC with a dual detection (UV and RI) [8]. Since H_2NPCL did not absorb in this region, the successful grafting was directly emphasized by SEC (Fig. 1). A new elution peak appeared at a lower elution volume (RI detection) compared to the original H_2NPCL and grew with the C_{60} consumption (Fig. 1A). In parallel, a new elution peak was recorded by UV detection at 340 nm (Fig. 1B), consisting of the grafting of the H_2NPCL chains onto C_{60}. However, at shorter retention times, the relative intensity of the UV trace is higher than the RI trace, which implies that the fullerene content is higher in these products. The same observation was previously reported for the SEC chromatograms of C_{60} grafted with polyoxypropylene chains [6]. Formation of aggregates was proposed as an explanation. Indeed, aggregation of stars with a smaller number of arms, i.e., derivatives rich in C_{60}, is favoured, and these aggregates thus exhibit a higher relative intensity for the UV-absorbance.

The average molar mass of the C_{60}–polyesters was determined by SEC (Table 1). The average number (x) of polyester branches per grafted C_{60} was calculated as the $M_{n,star(SEC)}$/$M_{n,arm}$ ratio and was found smaller than determined by elemental analysis (5 vs 6). The reason for this discrepancy has to be found by $M_{n,star(SEC)}$, which is an apparent (under-estimated)

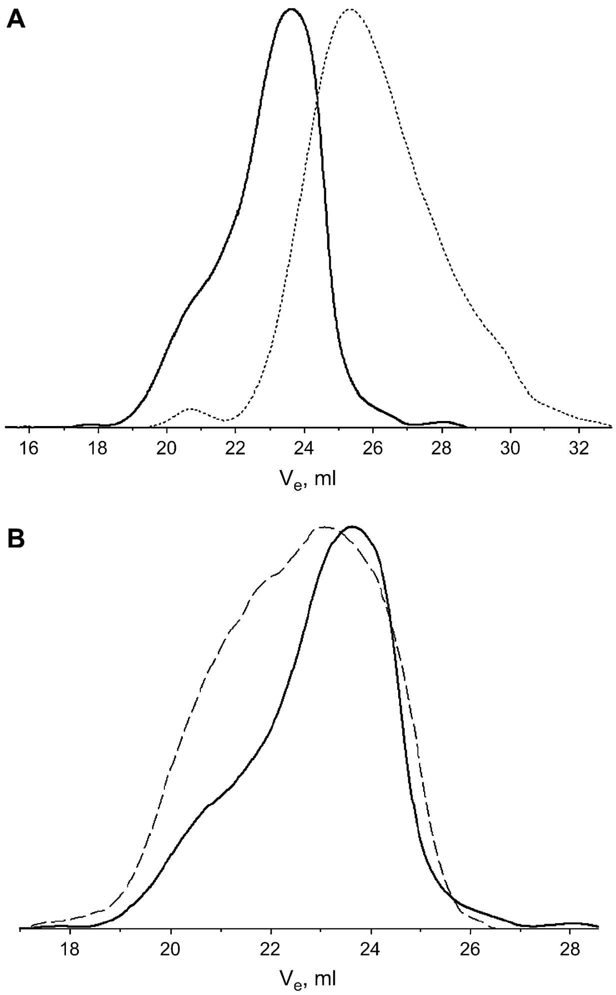


Fig. 1. SEC chromatograms. (A) RI traces of H_2NPCL_{31} (dotted line) and $H_xC_{60}(NHPCL_{31})_x$ (solid line); (B) RI (solid line) and UV (340 nm, dashed line) traces of $H_xC_{60}(NHPCL_{31})_x$.

Table 1
Molecular characteristics of H_2NPCL and the C_{60}–polyesters, and the average number (x) of PCL arms per fullerene core

Samples	Solvent	$\overline{M}_n$[a]	$\overline{M}_w/\overline{M}_n$[a]	x[b]
H_2NPCL_{31}	–	3700	2.51	–
$H_xC_{60}(NHPCL_{31})_x$	THF	21 000	1.55	5
$H_xC_{60}(NHPCL_{31})_x$	TCHB	19 000	2.18	5
H_2NPCL_{99}	–	11 500	2.50	–
$H_xC_{60}(NHPCL_{99})_x$	THF	55 000	1.54	5

[a] Determined by SEC.
[b] $x = M_{n,star(SEC)}/M_{n,arm}$.

value for a branched architecture [14]. Nevertheless, it is known that whenever an excess of low-molecular-weight amines is reacted with C_{60} an amine is attached to each of the six electron-deficient pyracylene units of C_{60} [15]. In this study, a 10-fold molar excess of amine-terminated polymer was used with respect to C_{60}, which gives credit to an average number of branches of six. The polydispersity of the grafted chains was lower when prepared in THF rather than in TCHB, which might be explained by the heterogeneity of the reaction medium in THF. Although the original H_2NPCL is of a high polydispersity, all the chains have the same probability of being grafted onto C_{60} under homogeneous conditions (in TCHB). In contrast, in a heterogeneous reaction medium, only $H_xC_{60}(NHPCL)_x$ products with enough grafted chains of a sufficiently high degree of polymerization ($\overline{DP}$) are soluble in THF. They are, therefore, recovered with a more uniform size distribution as a result of the fractionation of the PCL chains. This effect was confirmed by the higher average molar mass of the products formed in THF.

Comparison of the ^1H NMR spectra of the $H_xC_{60}(NHPCL)_x$ grafted products and the original H_2NPCL chains was an additional evidence for the success of the grafting reaction. Indeed, the ^1H NMR signal at 2.64 ppm, characteristic of the methylene protons next to the amine group ($-CH_2NH_2$), completely disappeared (Fig. 2).

Finally, the IR spectrum of the $H_xC_{60}(NHPCL)_x$ products was quite comparable to that of H_2NPCL. In the spectra of the grafted products, the NH_2 deformation band at 1590–1585 cm^{-1}, however, disappeared in agreement with the occurrence of the reaction between C_{60} and H_2NPCL. This observation was previously reported in the case of reaction of C_{60} with aminopolyethers [16].

3.2. Synthesis of C_{60}-containing star-shaped polyether, $H_xC_{60}(NHPEG)_x$

Although soluble in THF, the star-shaped $H_xC_{60}(NHPCL)_x$ chains were insoluble in water. In order to prepare water-soluble C_{60}-derivatives with an unexpanded structure, a commercially available monoaminopolyether (H_2NPEG) was reacted with C_{60} in THF (heterogeneous) and toluene (homogeneous), respectively. Expectedly, the reaction proceeded as observed with H_2NPCLs (Scheme 3). For instance, the colourless monoaminopolyether became brown in THF (heterogeneous reaction), and the violet homogeneous solution of C_{60} in toluene turned red-brown upon reaction with H_2NPEG. The $H_xC_{60}(NHPEG)_x$ grafted chains were soluble in THF in contrast to the unreacted C_{60} that was insoluble and easily separated by centrifugation and filtration. The reaction products were brownish solids and soluble in water and THF.

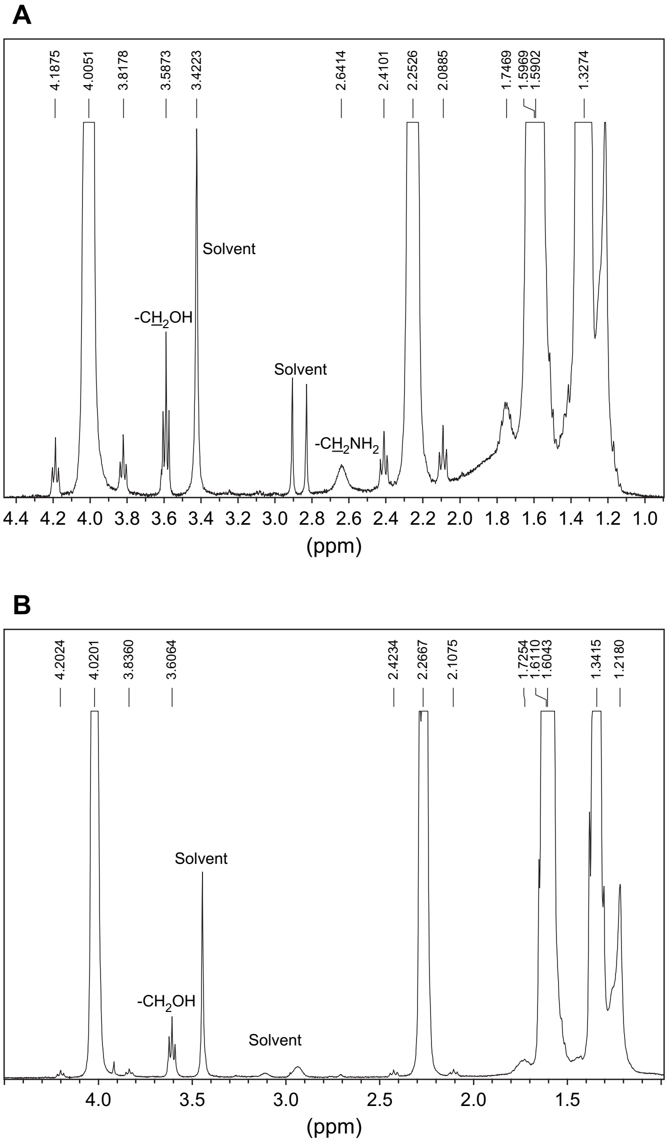


Fig. 2. ^1H NMR spectra of H_2NPCL_{31} (A) and $H_xC_{60}(NHPCL_{31})_x$ (B).

The SEC chromatograms of $H_xC_{60}(NHPEG)_x$ were illustrative of the successful grafting (Fig. 3). Indeed, $H_xC_{60}(NHPEG)_x$ was eluted at a lower elution volume than the original H_2NPEG (Fig. 3A). The apparent $\overline{M}_n$ was 8500 compared to 2000 for H_2NPEG, and the polydispersity was low ($\overline{M}_w/\overline{M}_n = 1.20$). Because only the C_{60}-containing chains could be detected by UV (at 340 nm), Fig. 3B was an additional evidence that $H_xC_{60}(NHPEG)_x$ was formed by reaction of H_2NPEG with C_{60}.

The average number (x) of polyether arms grafted onto a C_{60} core was calculated by elemental analysis and compared to SEC analyses. These data are listed in Table 2. Expectedly (cfr supra), x is smaller when determined by SEC compared to the absolute determination by elemental analysis. As previously

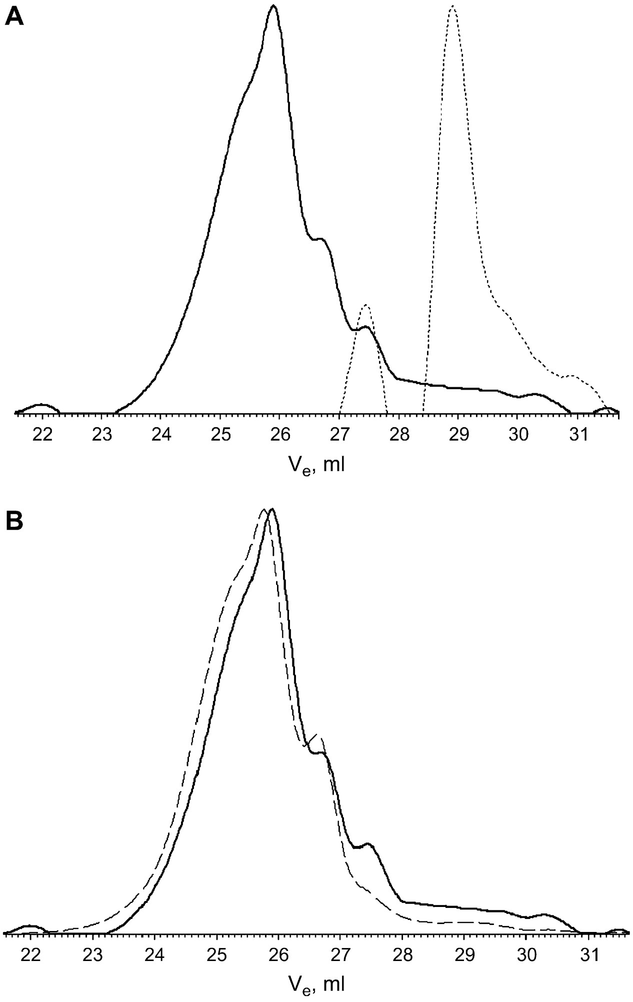


Fig. 3. SEC chromatograms. (A) RI traces of H_2NPEG (dotted line) and $H_xC_{60}(NHPEG)_x$ (solid line); (B) RI (solid line) and UV (340 nm, dashed line) traces of $H_xC_{60}(NHPEG)_x$.

observed in the case of H_2NPCL, the polydispersity of the star-shaped PEG chains was lower when prepared in THF rather than in toluene ($\overline{M}_w/\overline{M}_n = 1.20$ and 1.07, respectively).

Moreover, ^{13}C NMR signals at 41.69 and 72.58 ppm, characteristic of the methylene carbons next to the amine group ($-CH_2NH_2$ and $-CH_2CH_2NH_2$), completely disappeared in agreement with the successful grafting of the polyether chains onto fullerene (Fig. 4).

In a previous work, some of us reported that the thermal stability of PEG was improved by grafting onto C_{60} [6,17]. The thermal behavior of $H_xC_{60}(NHPEG)_x$ was thus investigated by thermogravimetric analysis as shown in Fig. 5. Once again, the C_{60} containing star-shaped PEG was thermally more stable than H_2NPEG. A very sharp weight loss was observed in the 350–400 °C temperature range, as a result of the polyether degradation. The non-volatile residue at 800 °C is the consequence of the accumulation of carbon and C_{60}.

Table 2
Molecular characteristics of H_2NPEG and the C_{60}-products, and the average number (x) of PEG arms per fullerene core

Samples	Solvent	$\overline{M}_n^{SEC}$[a]	$\overline{M}_n^{EA}$[b]	x^{SEC}[a]	x^{EA}[b]
H_2NPEG	–	2000	1700	–	–
$H_xC_{60}(NHPEG)_x$	Toluene	8500	10 700	4	6
$H_xC_{60}(NHPEG)_x$	THF	11 000	–	5	–

[a] Determined by SEC chromatography.

[b] Determined by elemental analysis.

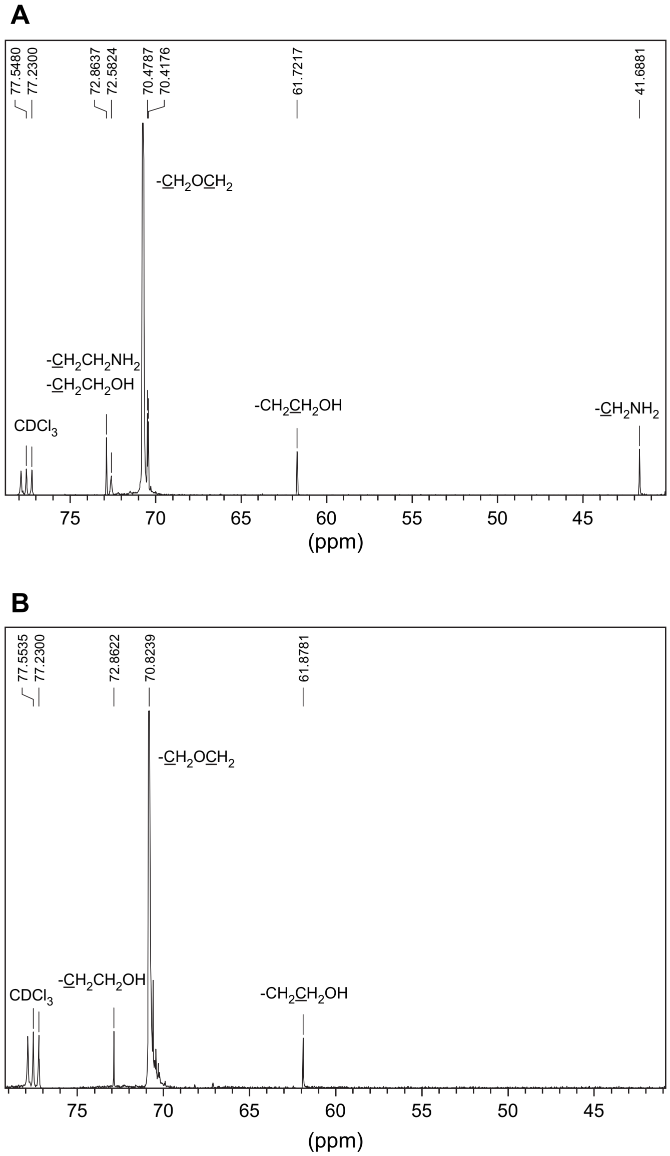


Fig. 4. ^{13}C NMR spectra (in $CDCl_3$) of H_2NPEG (A) and $H_xC_{60}(NHPEG)_x$ (B).

All the experimental observations confirmed the successful grafting of H_2NPCL and H_2NPEG chains onto C_{60}. Because fullerene is a potential singlet oxygen producer, attention was paid to this typical property for the herein-prepared star-shaped polymers.

3.3. Singlet oxygen generation by $H_xC_{60}(NHPCL)_x$ and $H_xC_{60}(NHPEG)_x$

It was previously reported that a reasonable quantity of singlet oxygen ($^1O_2$) was released upon photoactivation with

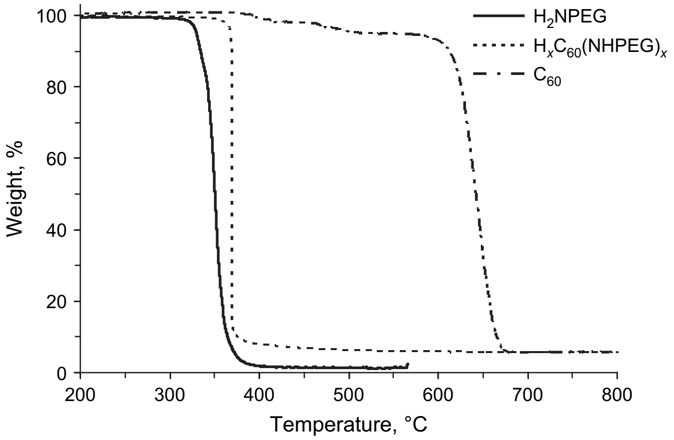
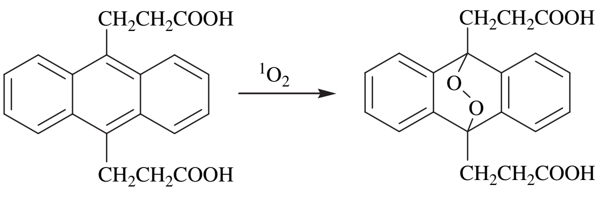


Fig. 5. TGA curves for initial H_2NPEG, C_{60}, and $H_xC_{60}(NHPEG)_x$.

visible light of C_{60}-containing PCL by the common ADPA bleaching method [8]. In this work, the photooxidation of ADPA into endoperoxide by $^1O_2$ was used to investigate the photoactivity of the $H_xC_{60}(NHPCL)_x$ and $H_xC_{60}(NHPEG)_x$ hybrids (Scheme 4).

Mixed $H_xC_{60}(NHPCL)_x$/ADPA solutions in THF were irradiated at a wavelength selectively absorbed by the photosensitizer (red filter). The reaction progress was recorded by monitoring the decrease of the ADPA absorption at 400 nm with the irradiation time (Fig. 6A). Control experiments in the absence of $H_xC_{60}(NHPCL)_x$ indicated that ADPA was stable towards irradiation at this wavelength (Fig. 6B). A significant amount of singlet oxygen was released upon irradiation of the $H_xC_{60}(NHPCL)_x$ solution for 2 h, as expressed by the decrease in the absorption intensity of ADPA (Fig. 6). The photosensitivity of these C_{60}—aminopolyesters with an unexpanded structure was compared to that of the previously studied, expanded counterparts, C_{60}—azidopolyesters, under the same experimental conditions. The absorption intensity at 400 nm decreased by ca. 50% in the case of 1 h irradiation of C_{60}—azidopolyester solutions, compared to only 24% in the case of C_{60}—aminopolyesters. Therefore, more singlet oxygen is produced by C_{60}-products with an unexpanded structure.

The photoactivity of the water-soluble C_{60}—aminopolyethers was also investigated by the same method at the same wavelength (400 nm). Moreover, the effect of a singlet oxygen quencher (NaN_3) and deuterium oxide (D_2O) on the photoactivity was considered (Fig. 7). The bleaching rate of ADPA photosensitized by $H_xC_{60}(NHPEG)_x$ was higher in deuterium oxide than in water. For example, the absorbance of an aqueous solution decreased by 37%, after 30 min irradiation compared to 85% in D_2O. This observation results from a difference in the singlet oxygen lifetime, which is ∼30 times



Scheme 4. Photooxidation of 9,10-ADPA.

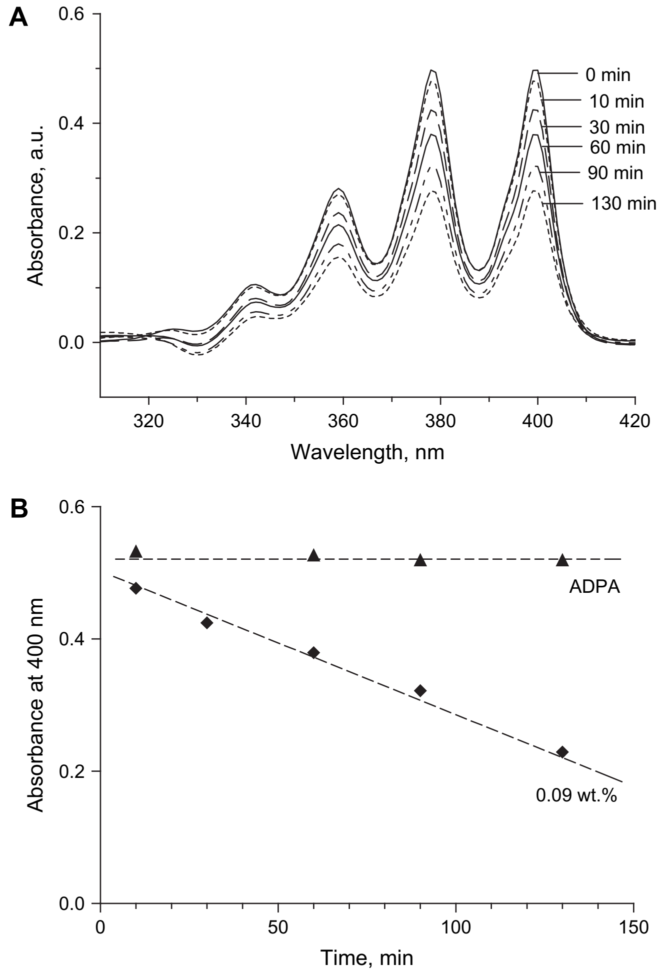


Fig. 6. Absorption spectra during the bleaching of ADPA by $H_xC_{60}(NHPCL_{31})_x$ (A), and absorbance of ADPA at 400 nm in THF as a function of the irradiation time (B).

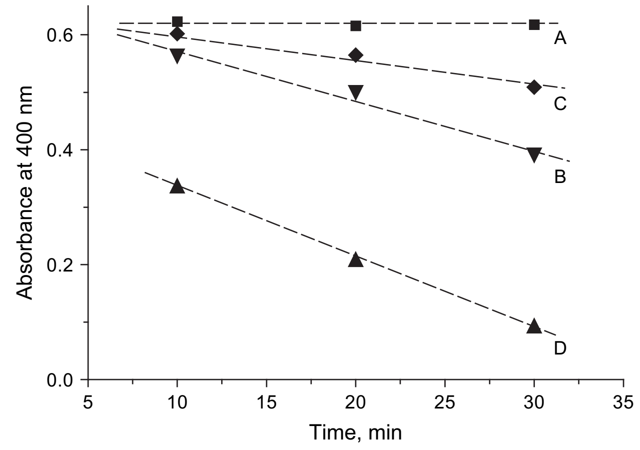


Fig. 7. Absorbance of ADPA aqueous solutions at 400 nm as a function of the irradiation time. (A) Blank control (ADPA in H_2O), (B, C, and D) in the presence of 0.13 wt% $H_xC_{60}(NHPEG)_x$, (C) with added NaN_3 (8.6 mM), (D) in D_2O instead of H_2O.

longer in D_2O than in water [18]. Sodium azide is an $^1O_2$ scavenger, that competes the $H_xC_{60}(NHPEG)_x$ photosensitizer, consistent with the experimental decrease in the bleaching rate of ADPA in the presence of NaN_3. Clearly, C_{60}–aminopolyethers are effective photosensitizers for $^1O_2$-mediated reactions and they are thus promising candidates for photodynamic cancer therapy. In this respect, the cytotoxicity of these C_{60}-derivatives must be addressed.

3.4. Cytotoxicity of $H_xC_{60}(NHPEG)_x$

The water-solubility of C_{60}–aminopolyethers allowed their cytotoxicity to be estimated, which was not the case for C_{60}–aminopolyesters. Cell viability was assessed in vitro towards THP-1 cells by Trypan Blue staining in the presence of C_{60}–polyether at two concentrations (10^{-4} and 10^{-5} M). Cytotoxicity was expressed in terms of cell viability compared to untreated cells (Fig. 8). As seen, in the presence of $H_xC_{60}(NHPEG)_x$ the number of cell survivors is almost the same as in the control sample (in the absence of C_{60}–polyether). Therefore, these preliminary results at the cellular level, are very promising with the prospect of killing tumor cells by irradiated $H_xC_{60}(NHPEG)_x$. This strategy, however, deserves further investigation.

3.5. Electrospinning of $H_xC_{60}(NHPEG)_x$

Nowadays, steadily increasing attention is paid to electrospinning as a simple method for the preparation of nanofibrous materials [19], useful for tissue engineering and drug delivery [20–23]. Recently, we successfully prepared for the first time micro- and nanosized C_{60}-containing fibers by electrospinning [8]. This was an incentive to test whether $H_xC_{60}(NHPEG)_x$ nanofibers could be electrospun. PCL was selected as an adjuvant because of biocompatibility having in mind devices to be used in contact with living organisms [24]. Moreover, PCL is known as an exception to the rule of polymer incompatibility. Last but not the least, PCL is easily electrospun and could thus improve the spinability of the polymer solutions [8]. Mixed solutions of $H_xC_{60}(NHPEG)_x$ and PCL in chloroform were thus prepared and electrospun. An SEM micrograph of the accordingly prepared micro-/nanosized fibers and their diameter distribution are shown in Fig. 9. A trimodal distribution was observed with an average diameter of 270, 820 and 1480 nm, respectively, the major population having an average diameter of 270 nm.

The concentration of a polymer solution well-suited to the continuous production of fiber can be calculated by a semi-empirical approach proposed by Shenoy et al. [25]. The concentration calculated for the electrospinning of fullerene-core star-shaped PCL proved, however, to be higher than experimentally required. The assumption was proposed that hydrophobic

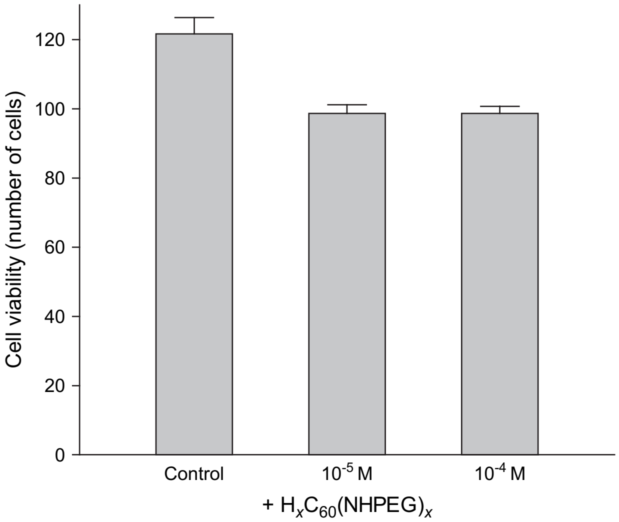


Fig. 8. Cell viability in the presence of $H_xC_{60}(NHPEG)_x$. Each assay was made in triplicate and the results are expressed as the average value ± standard deviation.

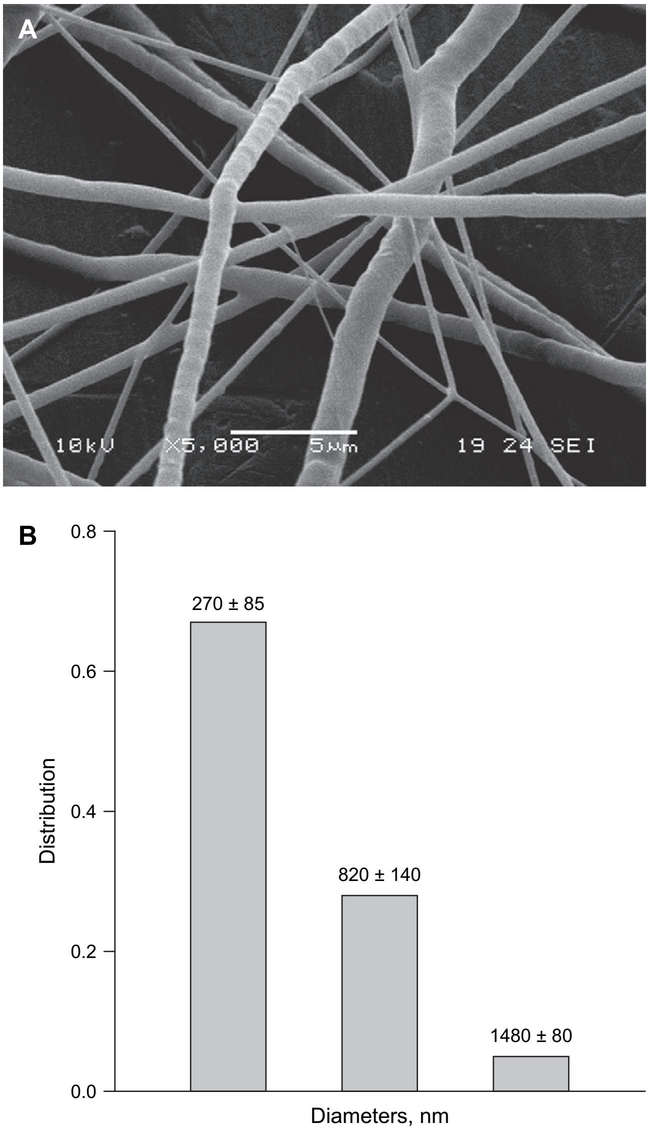


Fig. 9. SEM micrograph of electrospun mat of $H_xC_{60}(NHPEG)_x$/PCL (1/2, wt/wt) magnification ×5000 (A) and diameter distribution of the fibers (B). Applied field strength 1.0 kV/cm.

π–π interactions between fullerene cores contributed to stabilize a network of chains in addition to the chains' entanglements. In this study, the PCL concentration was 8%, i.e., lower than the calculated value for the continuous formation of PCL fibers (13%). This observation confirmed that contributions other than the physical entanglements of the chains are involved in the formation of the C_{60}-containing fibers.

4. Conclusions

In conclusion, star-shaped polyesters and polyethers with a fullerene core were successfully prepared by reaction of amino-terminated polyesters and polyethers with C_{60}. An average of six PCL or PEG chains per fullerene core was actually grafted. In contrast to C_{60}, the C_{60}–aminopolyesters and C_{60}–aminopolyethers were soluble in THF and in water, respectively. C_{60}-containing micro-/nanofibers were successfully prepared by electrospinning. Moreover, upon photoactivation, the prepared C_{60}-containing polymers effectively generate singlet oxygen, which makes them potential photo-chemotherapeutic agents and thus promising materials for photodynamic therapy of tumors and treatment of multidrug resistant pathogens.

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