[60]Fullerene and three [60]fullerene derivatives in membrane model environments

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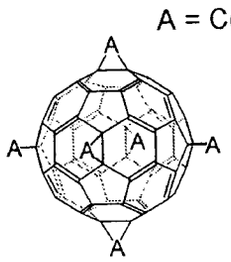
Aggregation of C_{60} and of three C_{60} derivatives in phosphatidylcholine liposome model membranes was studied *via* photophysical investigations. The triplet properties of these fullerenes, including the absorption spectrum between 400 and 900 nm, molar absorption coefficient, quantum yield of formation and/or quantum yield for the photosensitised production of $^1O_2$ were determined in benzene solution and in liposomes. One of the derivatives was a cyclopropyl-fused hexa-addended C_{60} containing as addend $C(CO_2C_{18}H_{37})_2$ (**1**). The other two derivatives were cyclohexyl-fused C_{60} with leucine (**2**) or hydrophilic acidic (**3**) mono-addend functionalisation. The synthesis of derivative **2**, by thermal extrusion of sulfur dioxide from the corresponding sulfone in the presence of C_{60}, is described. [60]Fullerene and derivative **1** show no T–T absorption in liposomes, although they do so in benzene solution (with a low triplet quantum yield for hexa-addended **1**). The absence of triplet–triplet absorption in liposomes is indicative of fullerene aggregation. By contrast, the amphiphilic mono-addend derivatives **2** and **3** present appreciable T–T absorption in liposomes, although less marked than in benzene solution. Mono-addend functionalisation therefore appears to be an interesting way to prevent or diminish fullerene aggregation. Moreover, in this type of derivatisation most of the photophysical properties of pristine [60]fullerene are retained.

Introduction

Use of fullerene-based compounds as drug candidates or drug vectors is under investigation by several groups.[1] In these initial studies, the high hydrophobicity of the all-carbon framework of fullerenes is usually circumvented by different methods of solubilisation including attachment of polar hydrophilic substituents[2–4] or association with amphiphilic organised structures such as micelles or lipid unilamellar vesicles[5,6] that are capable of merging with cell membranes and incorporate the fullerene compounds into the cell. These solubilisation methods can lead to fullerene aggregation[6] and thus to the appearance of solid phase properties that differ from those of the single molecule. Photophysical studies provide useful diagnostic tools to investigate dimerisation and aggregation of molecules.[6,7] In particular, it has been noted that singlet–triplet intersystem crossing quantum yields decrease as an effect of dimerisation or aggregation for aromatic hydrocarbons[8,9] and porphyrins.[10,11] Our preliminary results show a similar drop of the triplet quantum yield for C_{60} in phospholipid bilayer membranes.[12]

In the present work, we have extended this previous observation,[12] and compared the quantum yield of triplet formation (Φ_T) and/or singlet oxygen production (Φ_Δ) in organic solvents and in liposomes for C_{60} and for the three C_{60} derivatives **1**, **2** and **3**. For this comparison between photophysical properties of fullerenes in organic solvents and of fullerenes inserted in liposomes, we have used triplet data previously determined for C_{60}[13] and derivative **3**[14] and the results of our present determination of the triplet properties for compounds **1** and **2** which we have synthesised. Derivative **1** is an hexa-addend with long hydrocarbon chains which are expected to suppress contact between two neighbouring C_{60} cores and therefore prevent aggregation. Derivatives **2** and **3** each have one hydrophilic addend, but have low solubility in water; the addend is intended



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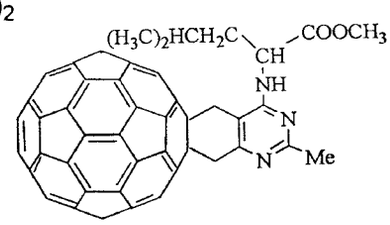

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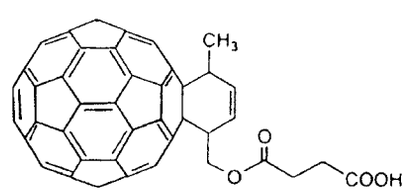

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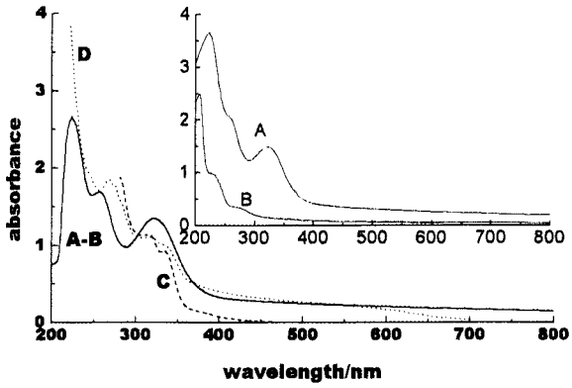


Fig. 1 Singlet ground state absorption spectra of the fullerene derivative **1** in benzene solution and incorporated in liposomes. A, compound **1** incorporated in liposomes; B, pure liposomes; A − B, difference spectrum; C, benzene solution of **1**; D, thin solid layer of **1**.

to render the compounds amphiphilic and aid their incorporation into liposomes.

Results and discussion

Ground state absorption

Absorption spectra of C_{60} and derivative 1 in liposomes and in benzene solution. The singlet ground state absorption spectrum of C_{60} in liposomes presents an additional absorption band in the visible region around 450 nm as compared to C_{60} in solution, interpreted as the result of the existence of fullerene aggregates formed in the hydrocarbon core of the bilayer.[6,12] The ground state absorption spectra of compound **1** in benzene and in liposomes are shown in Fig. 1. The shape of the absorption spectrum in benzene is similar to that already observed for multi-functionalised [60]fullerene compounds with its characteristic blue shift of the principal visible absorption bands with respect to C_{60}. The bands of **1** observed in benzene as small peaks at 317 and 334 nm (Fig. 1, curve C) are hidden in the spectrum of the liposome suspension by the light scattering behaviour of the latter medium (Fig. 1, curve A − B which represents spectrum A minus spectrum B, *i.e.* the difference between the spectrum of a suspension of liposomes incorporating the C_{60} derivative 1 and the absorption spectrum of fullerene-free liposomes). A broadening of the two spectral features in the 300–350 nm region as well as a red shift of these bands as compared to the benzene solution are considered as indicative of an aggregation process as for C_{60}. This explanation is supported by the observation of the same type of broadening in the ground state absorption spectrum of a thin solid layer of compound **1** (Fig. 1, curve D). We note however, for compound **1** in liposomes the spectral region corresponding to these two bands is preceded by a more pronounced valley than that observed for **1** in the solid state, and not present in the absorption spectrum of pure liposomes.

Absorption spectra of derivatives 2 and 3 in liposomes and in benzene solution. Fig. 2 (curve C) and 3 (curves C and D) show the ground state absorption spectra of compounds **2** and **3** in benzene solution and in liposomes (curves A), as well as the spectrum of pure phospholipid liposomes (curves B). The absorption spectra of these compounds in organic solvents (curve C) have as principal features a band peaking at 254 nm, a shoulder at 330 nm, a sharp band at 434 nm characteristic of [6,6]-closed ring bridged [60]fullerene, such as in dihydrofullerenes[14] and methanofullerenes,[15] and a final group of weak structures, the most intense of which peaks at 704 nm. The same features are also observable in the difference spectra shown in Fig. 2 and 3 (curves A − B). The overlapping of the ground state absorption of pure liposomes (curves B) is negligible at wavelengths longer than 350 nm.

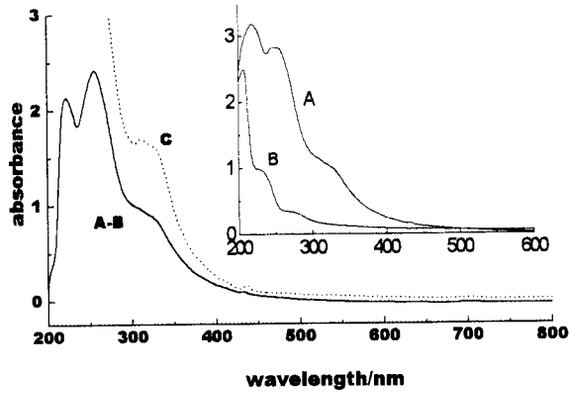


Fig. 2 Singlet ground state absorption spectra of the fullerene derivative **2** in benzene solution and incorporated in liposomes. A, compound **2** incorporated in liposomes; B, pure liposomes; A − B, difference spectrum; C, benzene solution of **2**.

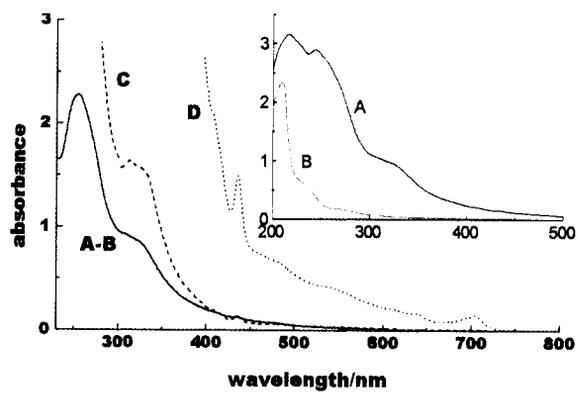


Fig. 3 Singlet ground state absorption spectra of the fullerene derivative **3** in benzene solution and incorporated in liposomes. A, compound **3** incorporated in liposomes; B, pure liposomes; A − B, difference spectrum; C and D, benzene solutions of **3** at concentrations of 5×10^{-4} and 5×10^{-5} M, respectively.

Triplet state absorption

Triplet state absorption spectra of C_{60} and derivative 1 in liposomes and in benzene solution. A small unstructured transient absorbance is observed between 400 and 900 nm after the laser excitation of an aqueous suspension of C_{60} in liposomes. This weak signal is not due to a triplet–triplet transition since it is not quenched by molecular oxygen.

No signal is observed in the case of liposomes incorporating hexaadduct compound **1**. However, in benzene it was possible to record the T–T absorption of compound **1** under the same excitation conditions (Fig. 4, curve 1). This spectrum of a hexaaddend derivative, with a maximum around 592 nm, resembles the T–T spectra of the mono-addended cyclopropyl- or cyclohexyl-C_{60} derivatives (maximum *ca.* 700 nm) but is more blue shifted relative to the C_{60} peak at 750 nm. The blue shift appears to depend on the number of opened double bonds with respect to C_{60} as has previously been observed for malonic ester C_{60} derivatives: $C_{60}[C(COOEt)_2]_n$.[16] The triplet lifetime in nitrogen saturated benzene solution was *ca.* 50 μs under our experimental conditions.

Triplet state absorption spectra of 2 and 3 in liposomes and in benzene solution. The T–T absorption spectra of compounds **2** and **3** incorporated in phospholipid liposomes are shown in Fig. 5 (compound **2**) and 6 (compound **3**). The T–T absorption spectrum for the liposomes which incorporate compound **2** presents the same spectral features, with λ_{max} *ca.* 692 nm, as the spectra in benzene solution obtained either by flash photolysis (Fig. 4, curve 2a) or by pulse radiolysis *via* sensitisation from the naphthalene triplet (Fig. 4, curve 2b). In each case the peak

Table 1 Triplet quantum yields Φ_T and molar absorption coefficients ε for compounds **1**–**3**[a]

Compound	Benzene		Liposomes in H_2O		Liposomes in D_2O
	$\Phi_T \approx \Phi_\Delta$	ε_T/M^{-1} cm^{-1}	$\Phi_T \approx \Phi_\Delta$	ε_T/M^{-1} cm^{-1}	$\Phi_T \approx \Phi_\Delta$
C_{60}	1[b]	20200[b] (λ = 750 nm)	0	—	
1	0.18	7800 (λ = 592 nm)	0	—	
2	0.70	18600 (λ = 692 nm)	0.66	4900 (λ = 692 nm)	0.57
3	1[c]	11000[c] (λ = 700 nm)	0.64	6200 (λ = 700 nm)	0.74

[a] Φ_Δ, Φ_T and ε_T values were determined with a ±15% precision; Φ_T values can be equated to the Φ_Δ values (see text). [b] Ref. 13. [c] Ref. 14, toluene solution.

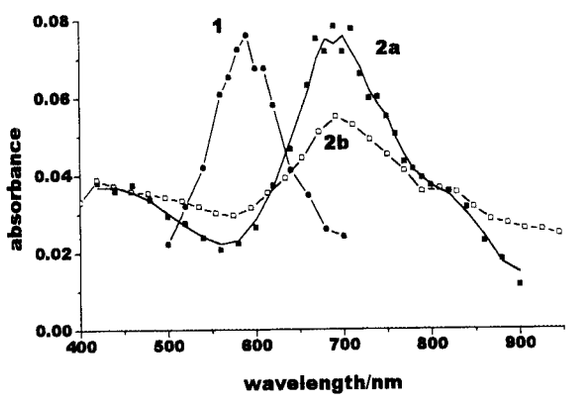


Fig. 4 Transient T–T absorption spectra of benzene solutions of derivatives **1** and **2**. 1, compound **1** (flash photolysis experiment); 2a, compound **2** (flash photolysis experiment); 2b, 5×10^{-5} M benzene solution of compound **2** sensitised by 1×10^{-2} M naphthalene (pulse radiolysis experiment).

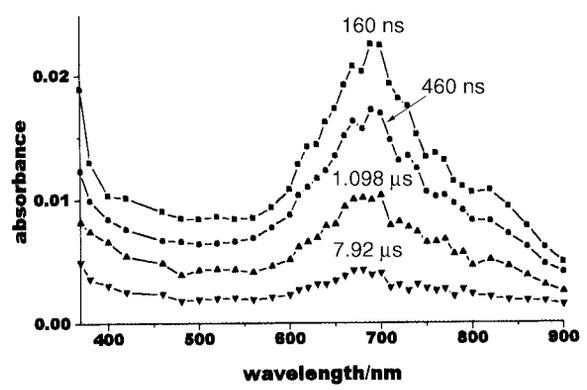


Fig. 5 Transient absorption spectra of an aqueous suspension of compound **2** incorporated in liposomes, recorded at different time intervals after a laser pulse excitation at 355 nm.

appears at 692 nm and this indicates the local environment to be non-polar in liposomes as in organic media. The T–T absorption spectrum for the liposomes which incorporate compound **3** is quite similar to that previously obtained with this compound in toluene solution[12] with λ_{max} *ca.* 700 nm. The shape of the spectrum was maintained with time, the decrease being due to the triplet state relaxation. Lifetimes of *ca.* 2.6 µs and *ca.* 2.0 µs were determined for compounds **2** and **3**, respectively. These lifetimes are somewhat lower than the lifetime of *ca.* 23 µs for compound **2** observed in benzene solution.

Determination of the quantum yield of singlet oxygen production of derivatives 1, 2 and 3 in benzene solution, and in liposomes prepared in H_2O and D_2O

The comparative method[17] has been used to determine the

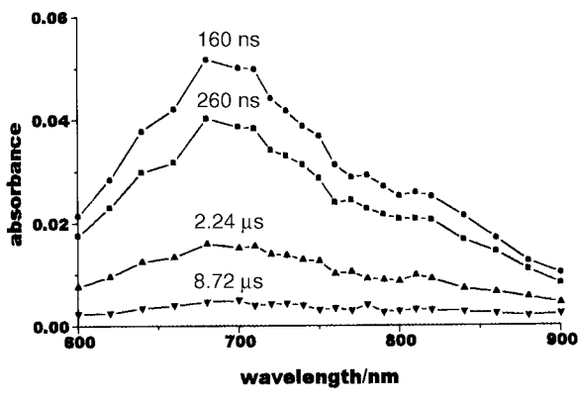


Fig. 6 Transient absorption spectra of an aqueous suspension of compound **3** incorporated in liposomes, recorded at different time intervals after a laser pulse excitation at 355 nm.

quantum yield of singlet oxygen production Φ_Δ according to eqn. (1):

$$\Phi_\Delta^F = (a_\Delta^F/a_\Delta^{St})(A_{\lambda exc}^{St}/A_{\lambda exc}^F)\Phi_\Delta^{St} \quad (1)$$

where a_Δ is the slope of the sensitised $^1O_2$ production as a function of pulse energy, $A_{\lambda exc}$ the absorbance of the solution at λ_{exc}, the wavelength of laser excitation, F and St refer to the fullerene derivative and to the standard sensitiser, respectively.

The oxygen phosphorescence data obtained at 1270 nm for the fullerene derivatives **1** or **2** or **3** in benzene solution or in liposomes prepared in H_2O or D_2O allowed us to determine the ratio a_Δ^F/a_Δ^{St}. The standard compounds were C_{60} in benzene solution, perinaphthenone for aqueous solutions and tetrakis-(4-sulfonatophenyl)porphyrin, $(H_2TPPTS)^{4-}$, for D_2O solutions. The Φ_Δ^{St} values used were unity for C_{60} in benzene,[13,18] unity for perinaphthenone in H_2O solutions[19] and 0.64 for $(H_2TPPTS)^{4-}$ in D_2O solution.[20]

Studies were carried out on oxygen-saturated suspensions of compounds **2** and **3** in liposomes prepared in D_2O instead of water, in order to increase the lifetime of the oxygen phosphorescence emission so as to determine more accurately the quantum yields Φ_Δ. The singlet oxygen phosphorescence lifetime was of the order of 17–18 µs for both compounds in D_2O–liposomes, whereas it was of the order of 65 µs when the singlet oxygen species are produced by the reference porphyrin compound in D_2O. This shortening of the oxygen phosphorescence lifetime, for liposomes prepared in D_2O as compared to pure D_2O, shows, less ambiguously than for experiments with liposomes prepared in water, that the singlet oxygen species experiences a micro-environment which is not completely aqueous. The Φ_Δ values determined for each compound in the different environments studied are given in Table 1.

Determination of the triplet quantum yield of compounds 1, 2 and 3 *via* Φ_Δ

The quantum yields of singlet oxygen production for compounds **1**, **2** and **3** allow us to estimate the corresponding Φ_T using the expression $\Phi_T = \Phi_\Delta(S_\Delta S_Q)^{-1}$ where S_Δ is the fraction of triplet photosensitiser molecules quenched by oxygen and leading to singlet oxygen $^1O_2$ ($^1\Delta_g$) and S_Q is the ratio of the sum of triplet decays involving oxygen over the overall triplet decay.[17b,21,22] The S_Q value is close to unity for **1**, **2** and **3** since the triplet lifetime decreases by two orders of magnitude in oxygen saturated solutions. The S_Δ value is considered to be unity for C_{60} [13,18] as well as for C_{60} methanofullerenes [15] since experimentally $\Phi_\Delta = \Phi_T$ for these molecules. In general the S_Δ value is 0.9 ± 0.1 for aromatic $\pi\pi^*$ triplet states.[23] Thus, if we assume the S_Δ value to be also unity for **1**, **2** and **3** in benzene and in liposome solutions, their Φ_T values can be considered as approximately equal to their Φ_Δ values (Table 1).

The lower value of Φ_T for derivative **1** compared to C_{60} is not surprising because the degree of functionalisation (opening of six double bonds) modifies the electron distribution, leading to a decrease in the rate of intersystem crossing as observed for malonic ester C_{60} derivatives $C_{60}[C(COOEt)_2]_n$.[16]

As for C_{60}, the non-detection of triplet state formation for compound **1** in the lipid bilayer is probably due to aggregation which leads to loss of triplet state conversion from the singlet excited state.[7–11] The aggregation of compound **1** and of another compound of analogous chemical structure (C_{12} instead of C_{18} hydrocarbon chains) in lipid bilayers has been observed by Hetzer *et al.*[24] from freeze fracture micrographs of dipalmitoylphosphatidylcholine multilamellar vesicles, where rod-like aggregates are formed in the inter space of the bilayer lipid sheets.

Determination of the triplet molar absorption coefficient ε_T *via* Φ_T

The molar absorption coefficient of the triplet state was determined using the comparative method [17] from the variation of T–T absorption intensity as a function of the laser pulse energy at the T–T absorption maximum. The value was computed using eqn. (2):

$$\varepsilon_T^F - \varepsilon_G^F = (\Phi_T^{St}/\Phi_T^F)(a_T^F/a_T^{St})(\varepsilon_T^{St} - \varepsilon_G^{St})(A_T^{St}/A_T^F) \quad (2)$$

where T, G, St represent triplet, ground state and standard compound, A and ε represent the absorbance and molar absorption coefficient, respectively, and a is the mean slope of the variation of the T–T absorption *vs.* the pulse light energy. The reference was a C_{60} solution in benzene. The calculated ε_T values for compounds **1**–**3** are given in Table 1.

Determination of the triplet molar coefficient ε_T *via* the oscillator strength [17c,d]

The T–T absorption of compound **2** is somewhat broader in liposomes than in benzene. According to the classical expression for oscillator strength (f) [eqn. (3)]:

$$f = \text{constant} \int \varepsilon(\nu)\mathrm{d}\nu \quad (3)$$

where $\int \varepsilon(\nu)\mathrm{d}\nu$ is the total area under the absorption band curve. If we assume that this oscillator strength f is not dependent on solvent then by equating the areas under the absorption curve of the T–T absorption band in benzene and in liposomes, replotted in a frequency scale between $\nu_1 = 11000$ cm^{-1} and $\nu_2 = 18000$ cm^{-1}, the maximum molar absorption coefficient of the T–T transition ε_T in liposomes was found to be 0.3 times the corresponding value in benzene (0.3×18600 M^{-1}/cm$^{-1} \approx 5600$ M^{-1} cm^{-1} at $\lambda = 692$ nm). This value is similar within 15% to that found *via* Φ_T ($\varepsilon_T = 4900$ M^{-1} cm^{-1}, Table 1), which justifies the assumption of solvent independence of f. The same calculation for compound **3** gives $\varepsilon_T = 0.65 \times 11000$ M^{-1} cm$^{-1} \approx 7150$ M^{-1} cm^{-1} at $\lambda = 700$ nm, which also is similar within 15% to the value found *via* Φ_T ($\varepsilon_T = 6200$ M^{-1} cm^{-1}, Table 1).

Conclusion

Going from benzene solutions to aqueous liposome suspensions decreases the quantum yields of triplet formation for C_{60}, and for compounds **1**, **2** and **3**. However, this decrease is smaller for the amphiphilic fullerene derivatives **2** and **3** than for C_{60} and compound **1**. This can be explained by a lower extent of aggregation for compounds **2** and **3**, since it is well established that specific deactivation, such as excimer formation, proceeds in aggregates [7–11] and decreases singlet to triplet intersystem crossing.

As a conclusion, the use of amphiphilic mono-addended derivatives of C_{60} in liposomes is an interesting route to reduce or prevent aggregation and as a result construct systems retaining most of the photophysical properties of fullerene itself.

Experimental

Materials

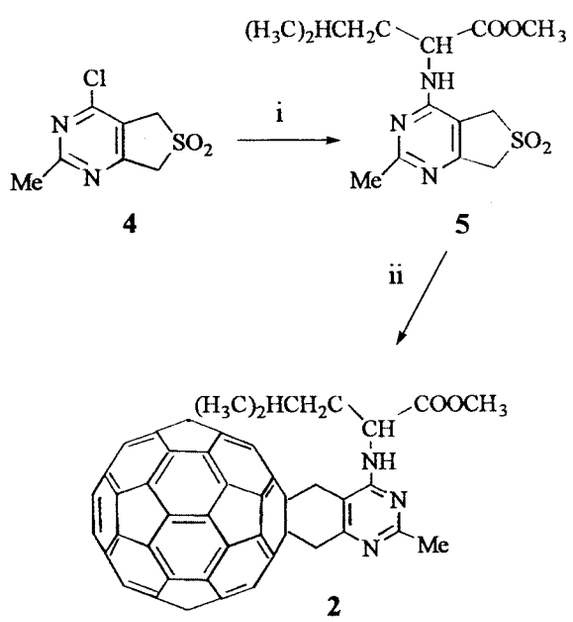
C_{60} derivative **3** was purchased from MER Corporation (Tucson AZ, USA), and L-α-phosphatidylcholine (PC) from Sigma (type XI-E, egg yolk lecithin). The synthesis of C_{60} derivative **2** is described below. C_{60} derivative **1** was synthesised according to the procedures described by Camps and Hirsch.[25]

Characterisation

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 spectrometer. Chemical shifts are quoted in ppm from (external) TMS; J values are given in Hz. Mass spectra (EI for sulfone **5**, LSIMS for compound **2**) were recorded on a VG AutoSpec-Q instrument. IR spectra were recorded on a Mattson 7020 Galaxy FTIR spectrometer. Melting points were determined with a Reichert Thermovar electric apparatus and are uncorrected.

Synthesis of compound 2 (Scheme 1)

The L-*N*-(fulleroquinazolin-4-yl)leucine derivative **2** was obtained by thermal extrusion of sulfur dioxide from sulfone **5**



Scheme 1 *Reagents and conditions*: i, $(CH_3)_2CHCH_2CH(NH_3^+Cl^-)$-$COOCH_3$, MeO^-–MeOH, r.t; **ii** C_{60}, 1,2,4-trichlorobenzene, 214 °C.

in the presence of C_{60}, as previously reported for similar sulfones.[26] Sulfone **5** was prepared by nucleophilic displacement of the chlorine atom in chloropyrimidine **4**[27] with L-leucine methyl ester.

Sulfone 5. The L-leucine methyl ester hydrochloride (831.2 mg, 4.58 mmol) was added to a solution of sodium methoxide (94.6 mg of Na, 4.11 mmol) in anhydrous methanol (10 ml) and the resulting suspension was stirred for 30 min. Sulfone **4** (100.0 mg, 0.46 mmol) was then added and the mixture was stirred for 4 days at room temperature and under nitrogen atmosphere. The solvent was removed by vacuum distillation, the residue was dissolved in water, the solution was acidified (pH = 3–4) and extracted with chloroform (3 × 15 ml). The organic extracts were dried with Na_2SO_4, the solvent was evaporated and the residue was purified by preparative TLC. Elution with chloroform–acetone (9:1) and crystallisation from diethyl ether–petroleum ether (bp 40–60 °C) yielded sulfone **5** (80%), mp 77–79 °C; ν_{max}(KBr)/cm^{-1} 2957, 2923, 2873, 1739, 1585, 1504, 1452, 1251, 1229; δ_H(300 MHz, $CDCl_3$) 0.97 (6H, d, *J* 4.5, CH_3), 1.62–1.74 (3H, m), 2.50 (3H, s, 2-CH_3), 3.78 (3H, s, OCH_3), 4.07 and 4.11 (2H, AB, *J* 15.3, CH_2SO_2), 4.25 and 4.28 (2H, AB, *J* 16.7, CH_2SO_2), 4.90–4.97 (m, C*H*NH), 5.30 (d, *J* 7.8, NH); δ_C(75 MHz, $CDCl_3$) 21.8, 22.8, 24.8, 26.0, 41.6, 51.9, 52.5, 53.3, 57.9, 104.7, 157.3, 157.6, 168.3, 174.5; *m*/*z* 327 ([M]$^{\bullet+}$, 13%), 284 (21), 271 (95), 268 (61), 263 (13), 220 (37), 207 (49), 204 (100), 160 (37), 147 (51), 135 (72), 119 (57), 107 (38).

Adduct 2. Sulfone **5** (15.0 mg, 0.046 mmol) and C_{60} (39.6 mg, 1.2 equiv.) were heated in refluxing 1,2,4-trichlorobenzene (7 ml), under nitrogen atmosphere, for 3 h. After cooling to room temperature, the mixture was purified by column chromatography. The trichlorobenzene and the unreacted C_{60} were eluted with petroleum ether (bp 40–60 °C) and the adduct was then eluted with chloroform (47%), mp > 310 °C; λ_{max}($CHCl_3$)/nm 702, 433, 310, 256; ν_{max}(KBr)/cm^{-1} 2951, 2920, 1745, 1581, 1429, 1151, 766, 575, 526; δ_H(300 MHz, $CDCl_3$–CS_2) 0.94–0.97 (6H, m, $(CH_3)_2$), 1.69–1.83 (3H, m, $CH_2CH(CH_3)_2$), 2.67 (3H, s, 2-CH_3), 3.75 (3H, s, OCH_3), 4.39 (2H, s, CH_2), 4.55 and 4.61 (2H, AB, *J* 13.8, CH_2), 5.06–5.14 (1H, m, C*H*NH), 5.39–5.45 (1H, m, NH); δ_C(75 MHz, $CDCl_3$–CS_2) 22.1, 22.9, 25.2, 26.2, 37.3, 41.7, 46.3, 52.0, 52.1, 64.8, 65.3, 109.9, 135.4, 135.7, 140.0, 140.1, 141.5, 141.6, 141.9, 142.0, 142.5, 143.0, 143.1, 144.5, 144.7, 144.8, 145.0, 145.1, 145.2, 145.4, 145.5, 145.6, 145.7, 146.1, 146.2, 146.5, 147.6, 155.2, 155.5, 155.7, 155.8, 158.1, 163.9, 166.4, 174.2; *m*/*z* 984 [M+H]$^+$, 720 [C_{60}^+].

Singlet ground state absorption spectra

The absorption spectra of C_{60} and compounds **1**, **2** and **3** in benzene solutions and incorporated in liposomes were recorded with a Kontron model Uvikon 940 spectrophotometer in the range 250–750 nm with a bandwidth of 1 nm and a data interval of 1 nm using a cell of 1 cm optical pathlength.

Transient absorption spectroscopy measurements

The T–T difference spectra were obtained either from benzene solutions of the compounds or from aqueous suspensions of the compounds inserted in liposomes, by direct excitation at 355 nm with a single 15 ns pulse of a Nd:YAG laser.[28] For the experiments in D_2O solutions the excitation was at 532 nm with a 6 ns pulsed YAG laser. The quantum yields of triplet state production as well as singlet oxygen formation were obtained by comparative measurements under the same medium.[17a,b] The values of the oxygen phosphorescence as well as the values of the triplet–triplet absorption were linear as a function of the laser pulse excitation energy in the 0.3–2.3 mJ energy range studied. A liquid nitrogen cooled germanium photodiode and an amplifier supplied by Applied Detector Corporation (USA) were used for the observation of the singlet oxygen phosphorescence at 1270 nm.

Pulse radiolysis

The pulse radiolysis set-up, based on a 9-12 MeV Vickers linear accelerator was similar to that described by Butler *et al.*[29] The T–T absorption experiments using pulse radiolysis were carried out in benzene solutions of the fullerene derivative and naphthalene. The formation of the excited states of naphthalene occurs by energy transfer from the solvent which absorbs the ionising radiations. The absorption spectrum of the fullerene species in its lowest triplet state was obtained by energy transfer from the triplet state of naphthalene the concentration of which (0.01 M) was much greater than that of the fullerene derivative (*ca.* 1 × 10^{-5} M).

Liposomes

The liposomes were prepared as follows. A rotary evaporator was used to dry a solution containing 20 mg L-α-phosphatidylcholine (PC) and a C60 derivative in a 3–4% mol/mol ratio in chloroform. A phosphate buffer solution (0.1 M NaCl–0.01 M KH_2PO_4–NaOH at pH 7) was prepared, and 2 ml of this buffer was added to the above mixture which was vortexed in the presence of glass beads. A titanium probe sonicator (Ultrasons Annemasse) was used to clarify the suspension. The sonication was carried out on samples in a water bath for several successive periods of 10–20 min, at 75–100 W, with rest periods of approximately 10 min between each sonication. The total sonication time was 40–60 min for the derivatives and 2 h for C_{60}. The samples were then centrifuged at 8500 g for 30 min (Heraeus Biofuge primo). Just before use the samples were filtrated on Millipore Millex-GV_{13} filter units (0.22 μm pore size). The various vessels were washed in order to evaluate the quantities of the derivatives which were not incorporated into the liposomes. The incorporation yield diminished in the order **3** > **2** > **1** > C_{60}. The final derivative/lipid molar ratio was 2.6% for **3**, 2% for **2**, 1.4% for **1** and 0.4% for C_{60}. The liposomes incorporating the derivatives showed peaks at 250 ± 5 nm and peaks or shoulders at 330 ± 10 nm, which do not appear for L-α-phosphatidylcholine liposomes made without any added derivative. The absorbance in the region of the triplet absorption was negligible.

Two separate methods and devices were used for measurement of the vesicles diameter and control of their size distribution by light scattering. In a set of experiments a Dawn DSP apparatus was used and the mean radius was obtained from a Zimm plot. For all the liposome samples the mean radius was 40 ± 5 nm. In the second type of experiment, the size of the vesicles was measured by a Sematech light scattering apparatus used at various diffraction angles in the correlation mode. Whatever the angle value, the size distributions were bimodal even after filtration of the liposome samples. The first distribution maximum is centered around 38.4 nm, a value not very different from the values obtained from the Zimm plot analysis, and presumably corresponds to a population of unilamellar vesicles, *i.e.* liposomes, the second maximum was of the order of 183 nm (40% weight) that we attributed to multilayered vesicles or to fused liposomes.

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