

**SYNTHESIS AND APPLICATIONS OF [60]FULLERENE NANOCONJUGATE
WITH 5-AMINOLEVULINIC ACID AND ITS GLYCOCONJUGATE AS DRUG
DELIVERY VEHICLES**

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ELEMENTAL ANALYSIS OF C₆₀-ALA

REACTION SCHEME FOR SYNTHESIS OF C₆₀-ALA and C₆₀-ALA-GA

¹³C-NMR of C₆₀-ALA

ESI-MS SPECTRUM OF C₆₀-ALA

UV-VIS SPECTRUM OF C₆₀-ALA-GA

DLS AND ZETA POTENTIALS OF C₆₀-ALA and C₆₀-ALA-GA

HPLC MEASUREMENTS OF PpIX IN MCF-7 CANCER CELL LINE

FLUORESCENCE SPECTRUM OF C₆₀-ALA

XPS STUDIES OF C₆₀-ALA-GA

SEQUENCES OF PRIMERS USED FOR RT-PCR

Element	Concentration
C	54.83
O	36.1
N	5.45
H	3.62

Table S1

Elemental analysis of fullerene C₆₀-ALA.

C₆₀(NHCH₂COCH₂CH₂COOH)_x(OH)_y EMPIRICAL FORMULA

$$N/C \text{ RATIO: } \frac{14X}{720 + 60X} = \frac{5.45}{54.83} \quad X=8.90 \approx 9 \text{ UNITS OF 5-ALA ATTACHED to C}_{60}$$

EMPIRICAL FORMULA: C₁₀₅H₉₆N₉O₅₁ (M_w= 2298 Da)

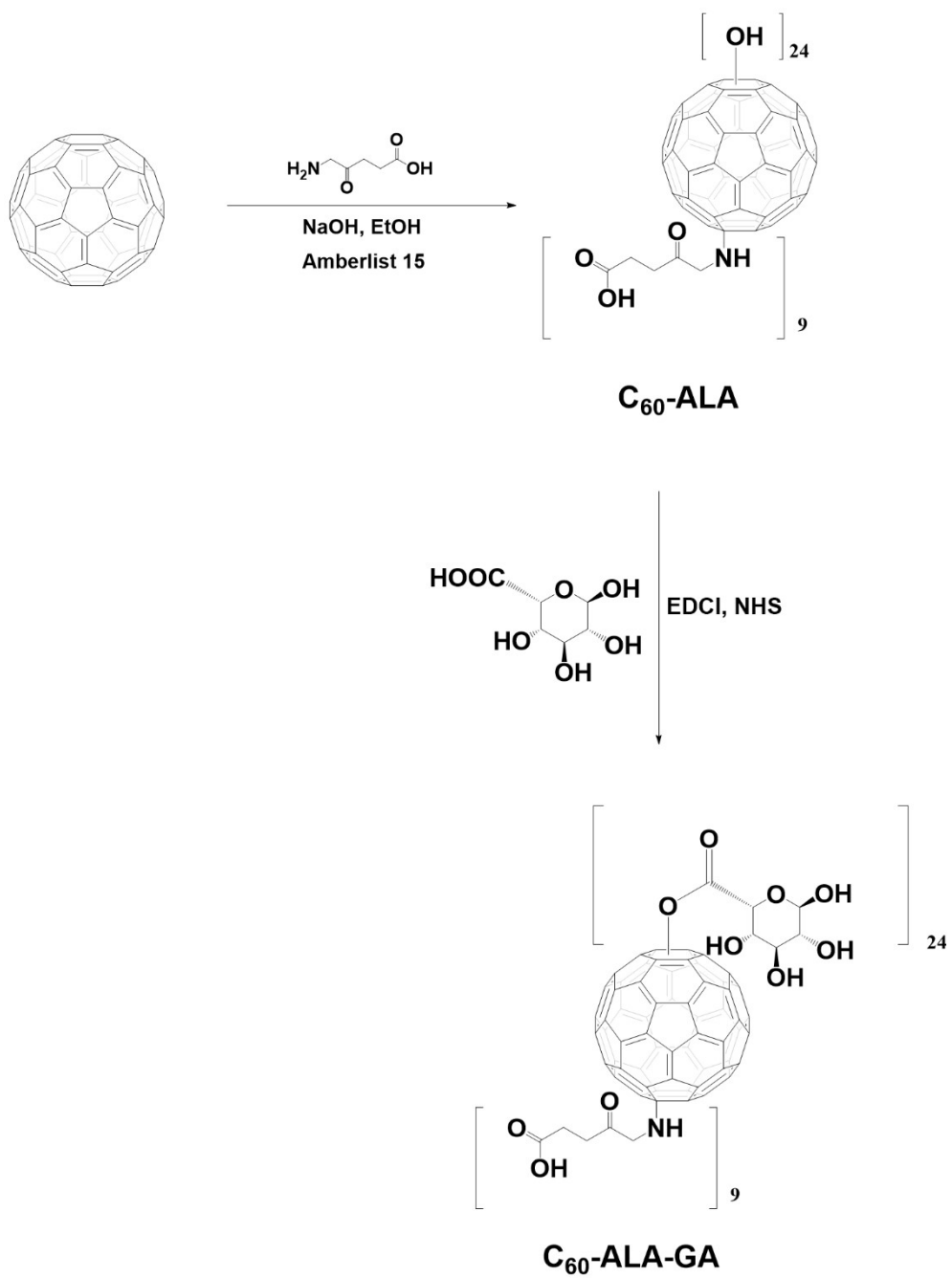


Figure S1

Synthetic plan for obtaining [60]fullerene nanomaterials $\text{C}_{60}\text{-ALA}$ and $\text{C}_{60}\text{-ALA-GA}$

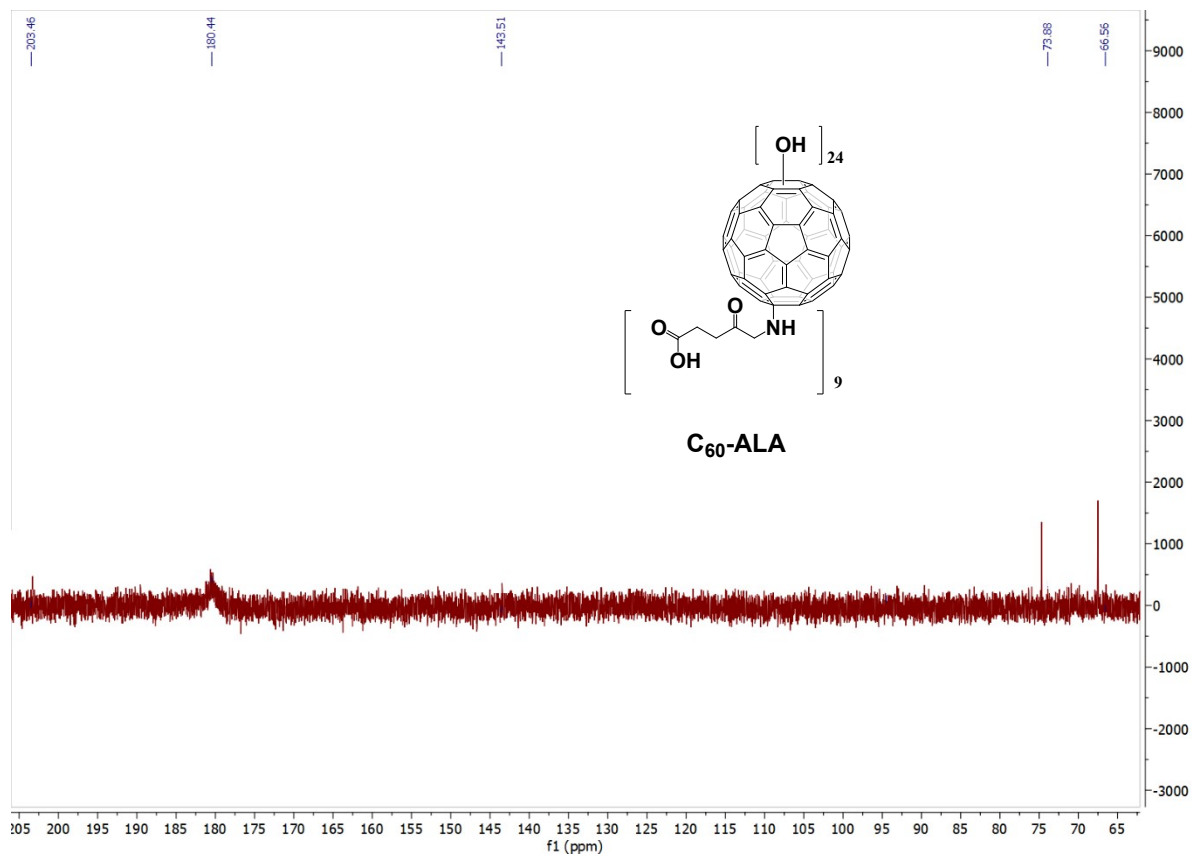


Figure S2

Fragment of ^{13}C -NMR spectrum of fullerene nanomaterial $\text{C}_{60}\text{-ALA}$

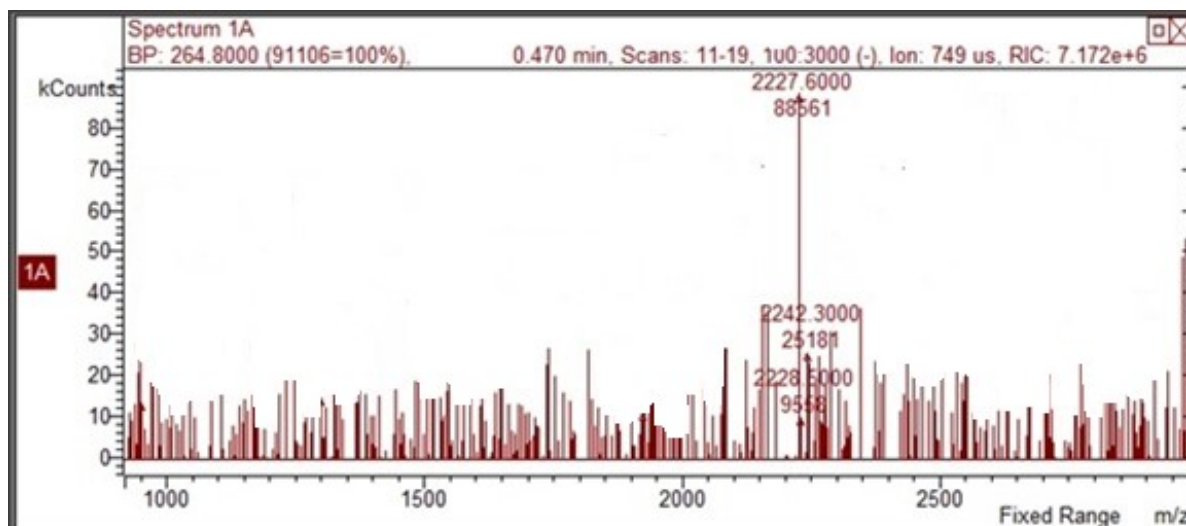


Figure S3

ESI-MS spectrum of fullerene nanomaterial C₆₀-ALA in a positive mode (100 mV).

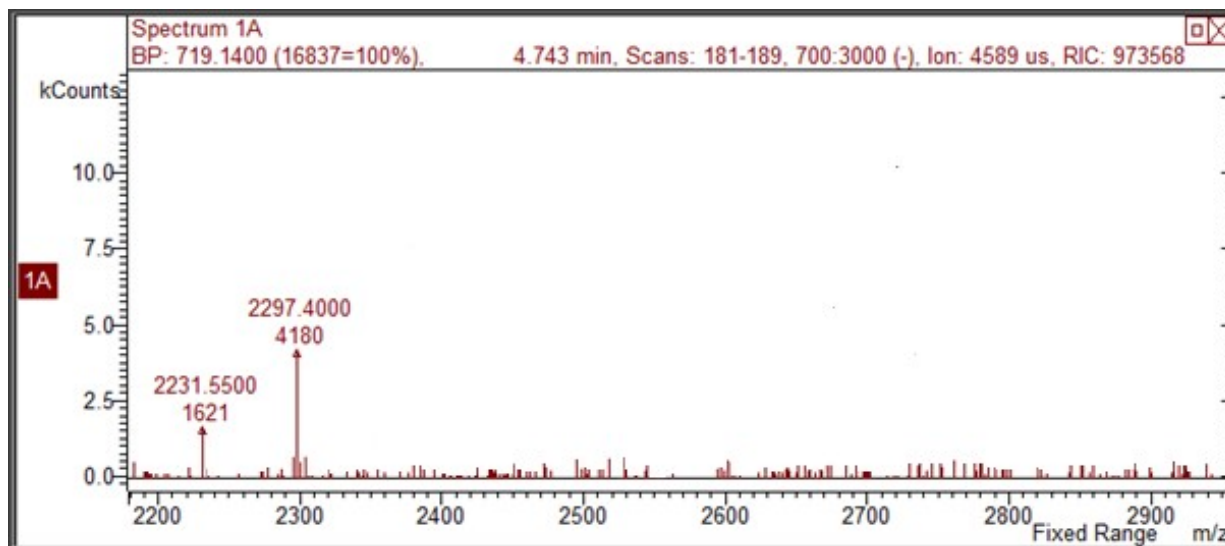


Figure S4

ESI-MS spectrum of fullerene nanomaterial C₆₀-ALA in a negative mode (25 mV).

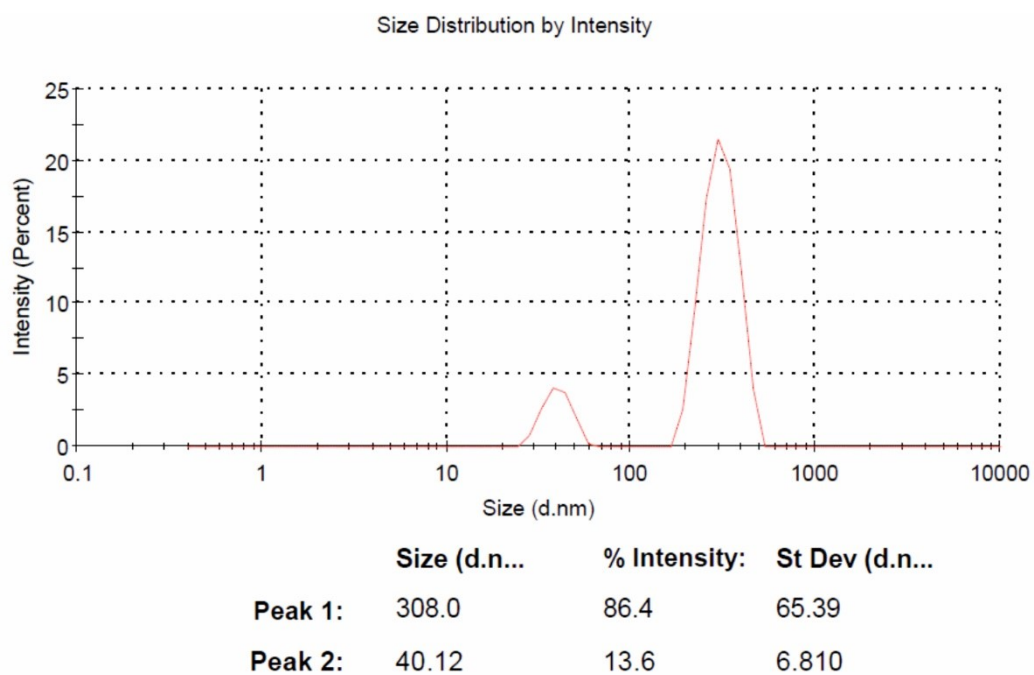


Figure S5

Size measurement of fullerene nanomaterial C₆₀-ALA using dynamic light scattering technique.

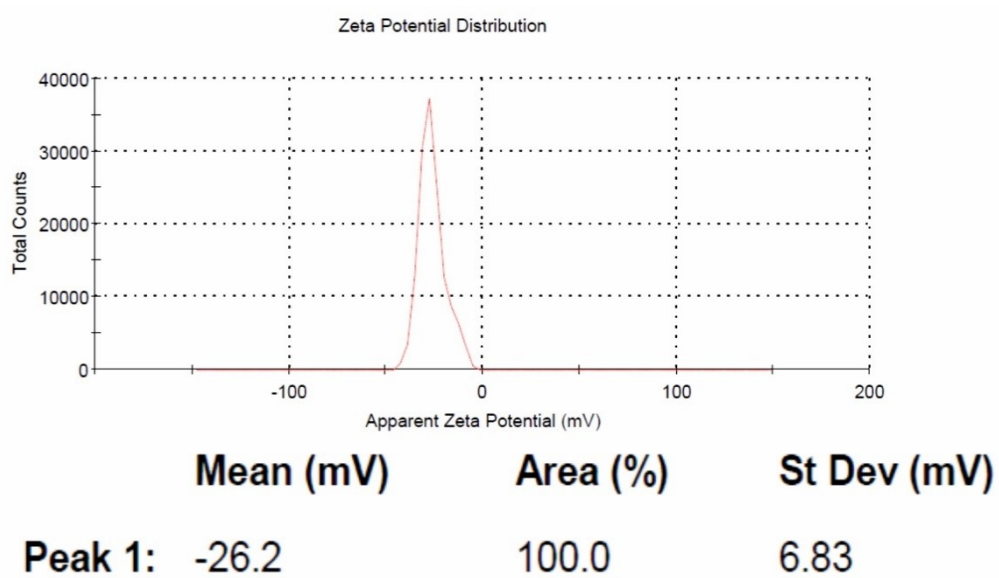
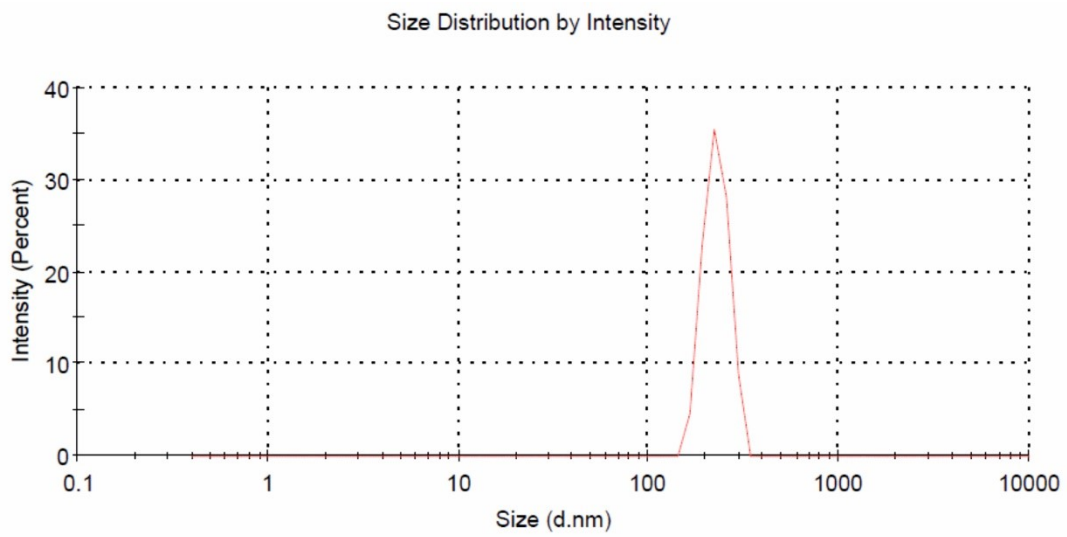


Figure S6

Zeta potential of fullerene nanomaterial C₆₀-ALA.



	Size (d.n...	% Intensity:	St Dev (d.n...
Peak 1:	227.1	100.0	33.83

Figure S7

Size measurement of fullerene nanomaterial C₆₀-ALA-GA using the dynamic light scattering technique.

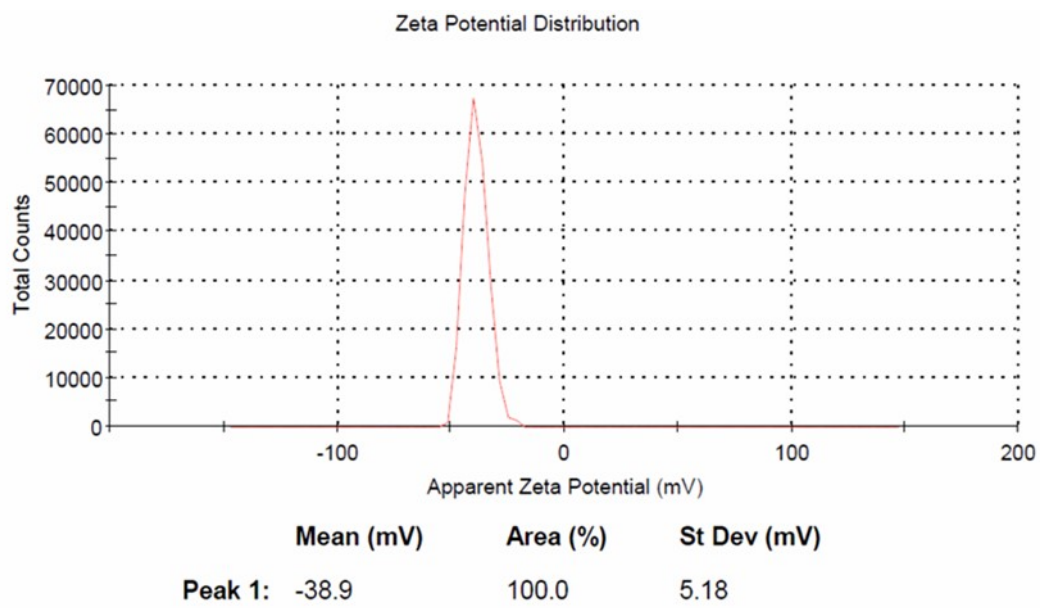


Figure S8

Zeta potential of fullerene nanomaterial C₆₀-ALA-GA.

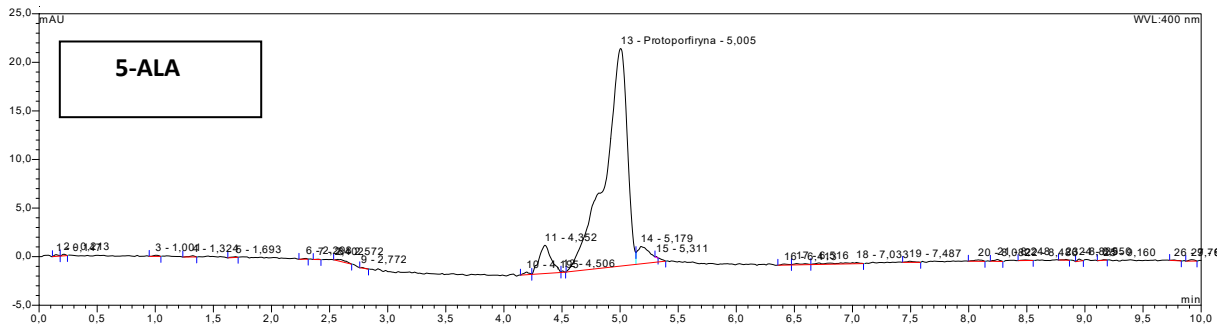
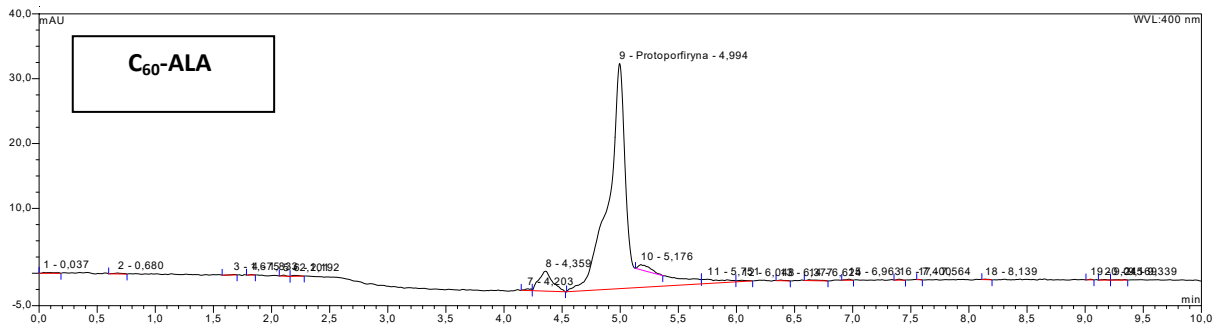
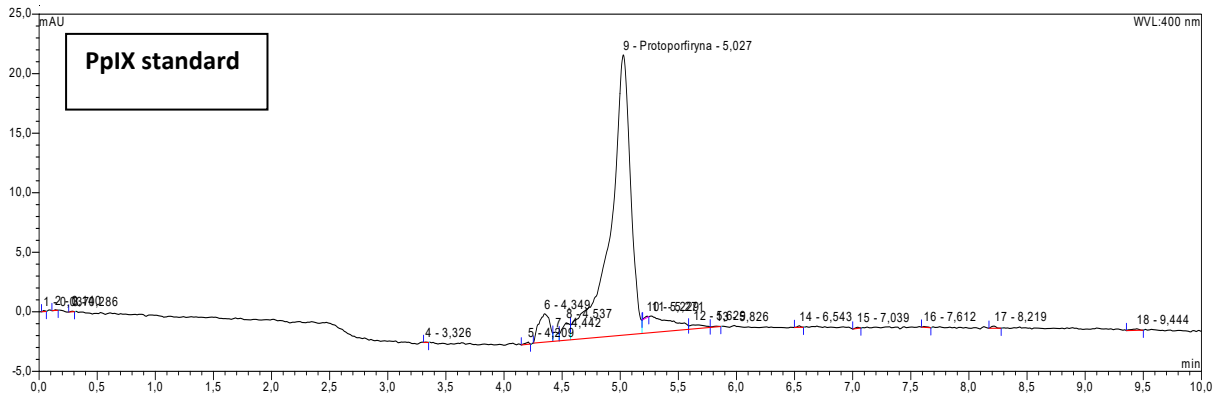
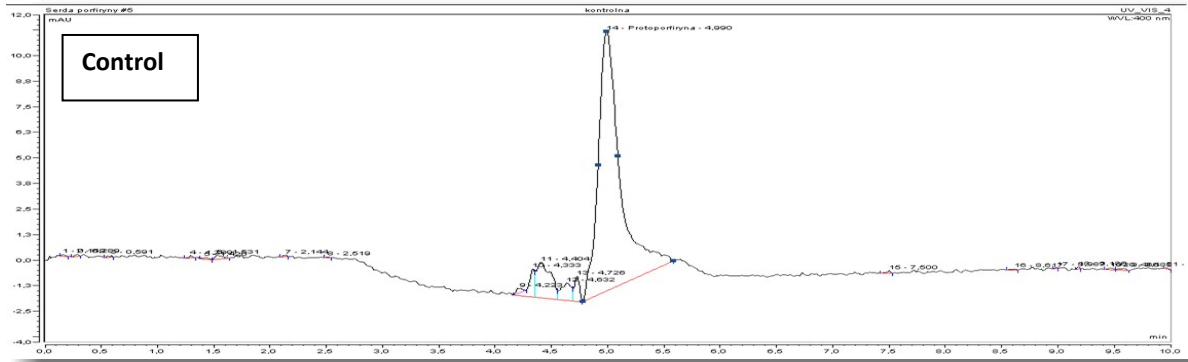


Figure S9

The HPLC chromatograms measured for dedicated bio-extracts from MCF-7 cancer cell line and standard (PpIX, Sigma Aldrich). The cells before extraction were untreated (control) or treated with 5-ALA, or fullerene nanomaterial C₆₀-ALA.

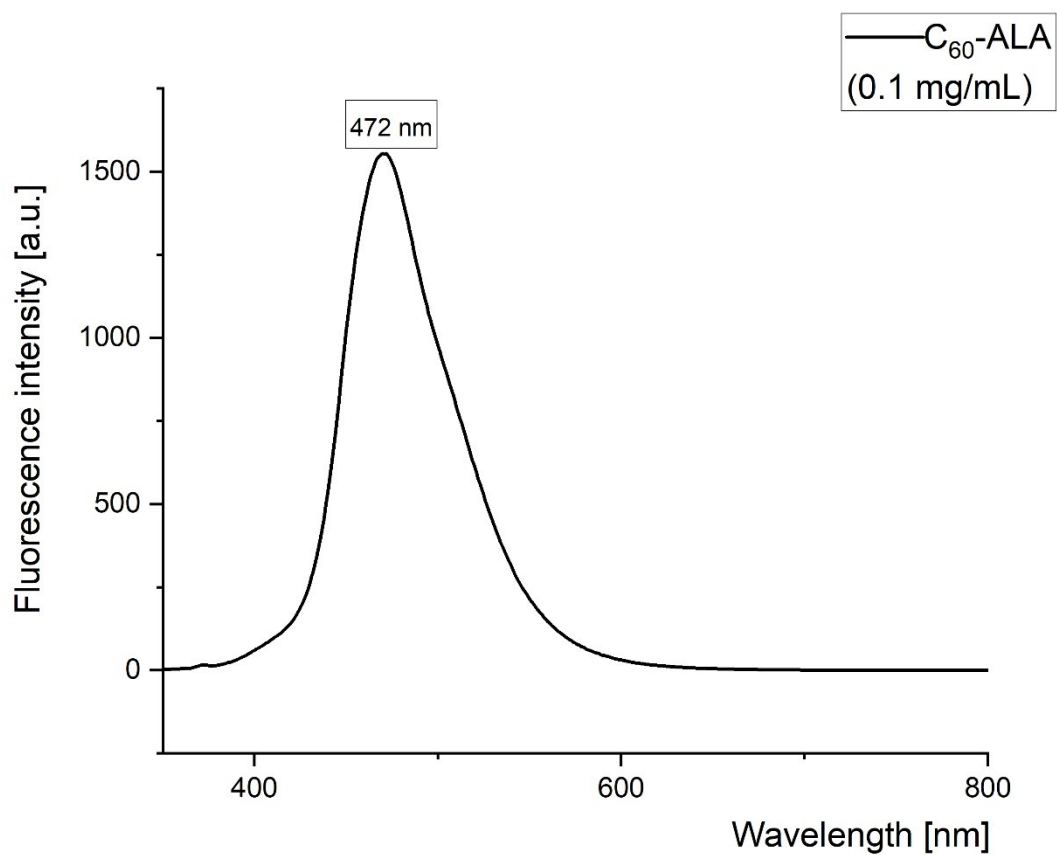


Figure S10

Fluorescent spectrum of C₆₀-ALA in water (c=0.1 mg/mL) excited at 340 nm.

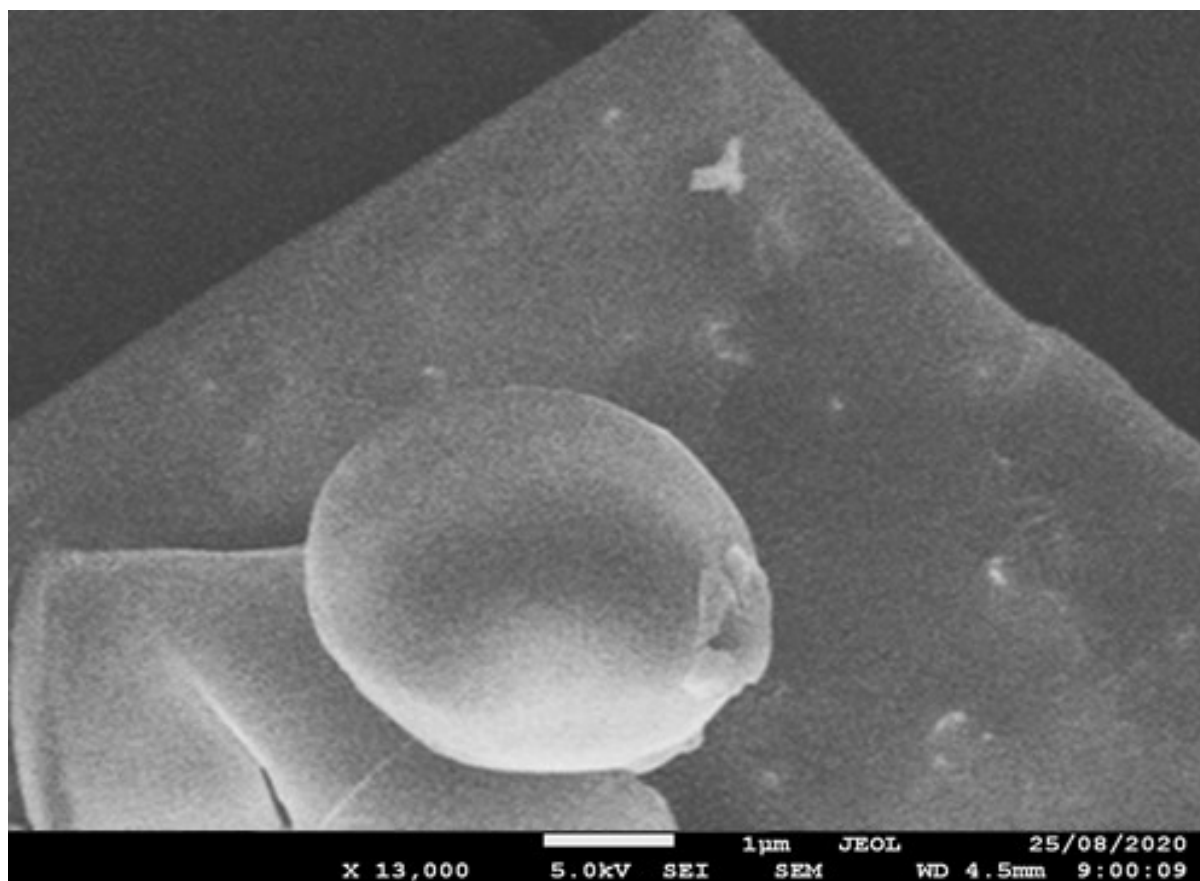


Figure S11

SEM image of fullerene nanomaterial C₆₀-ALA confirming its hollow structure.

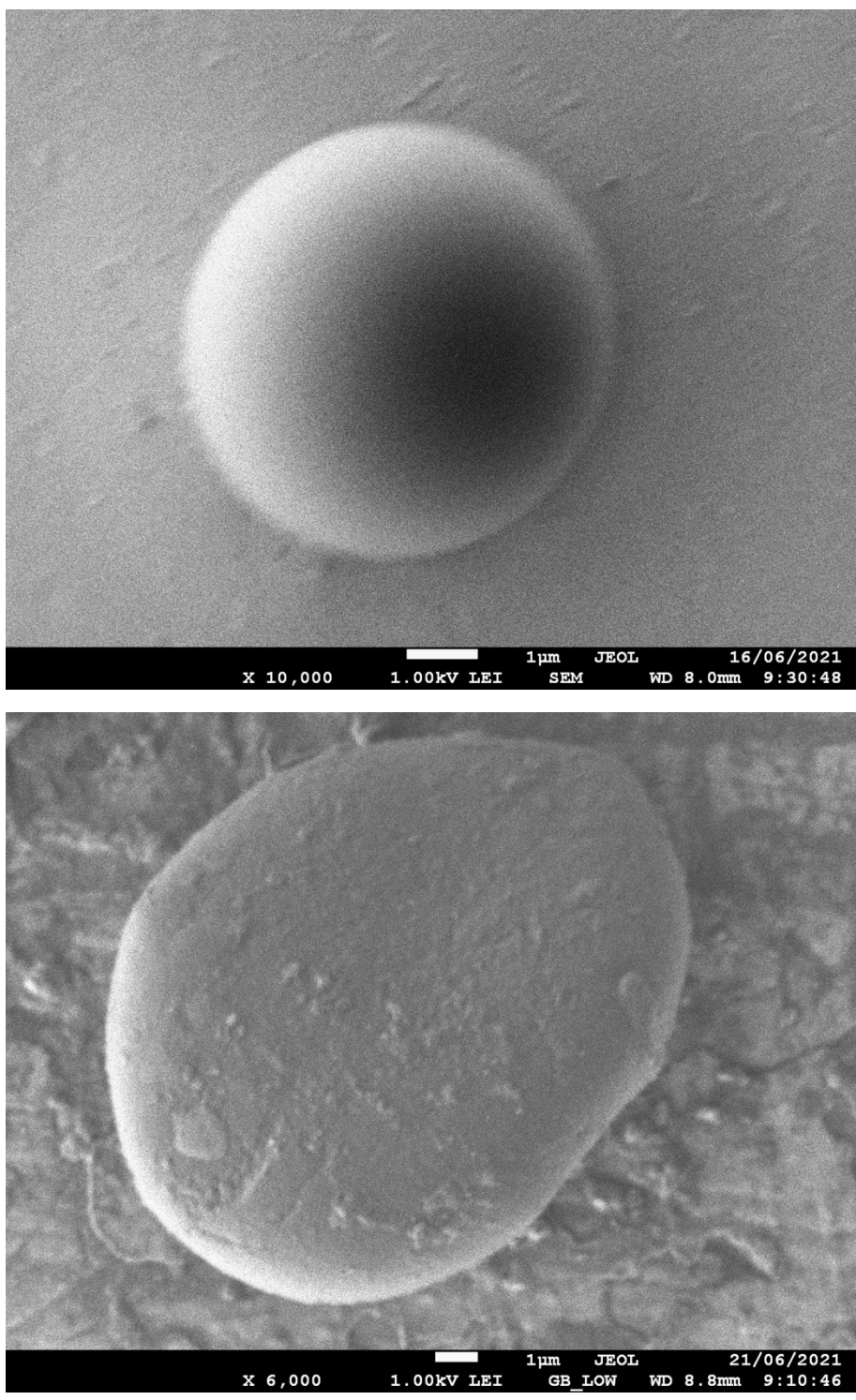


Figure S12

SEM images of fullerene nanomaterial C₆₀-ALA-GA.

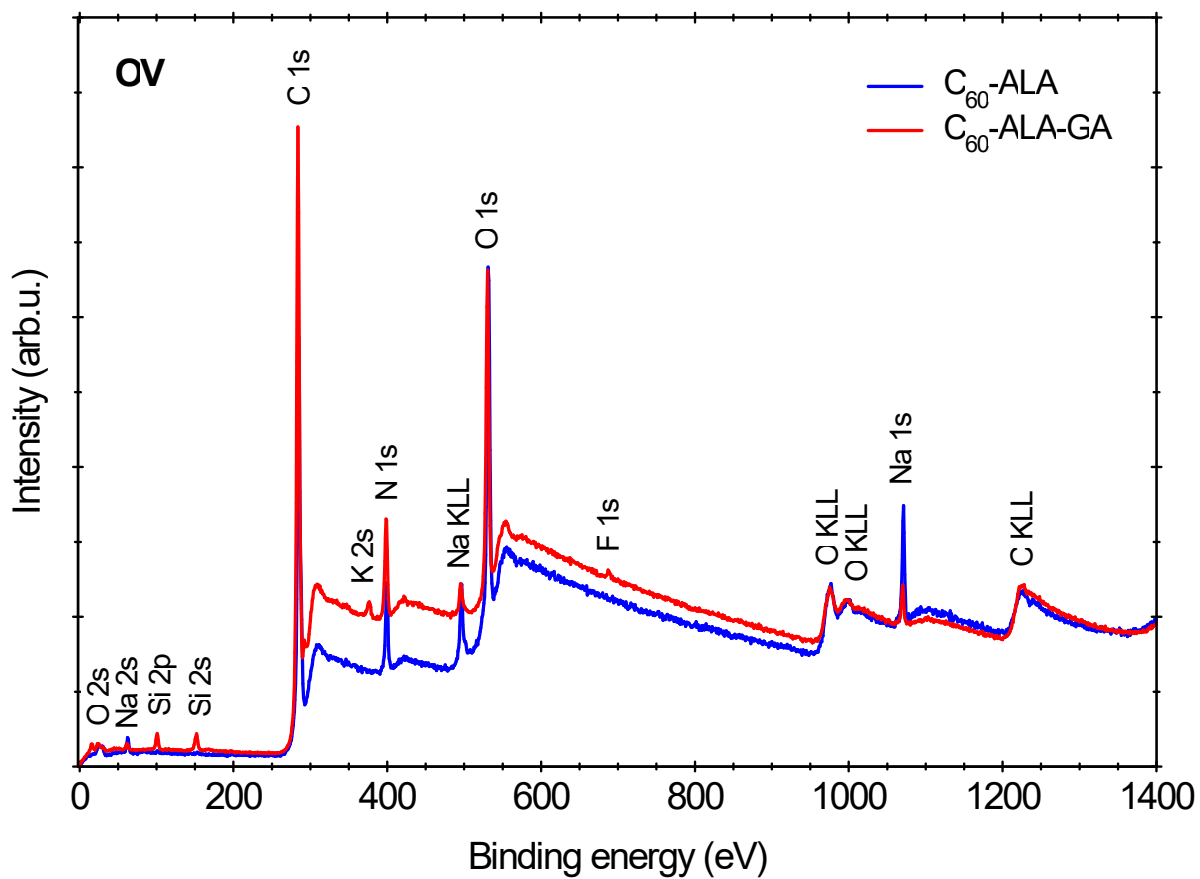


Figure S13

The XPS spectra of two fullerene nanomaterials in the wide energy range 0–1400 eV.

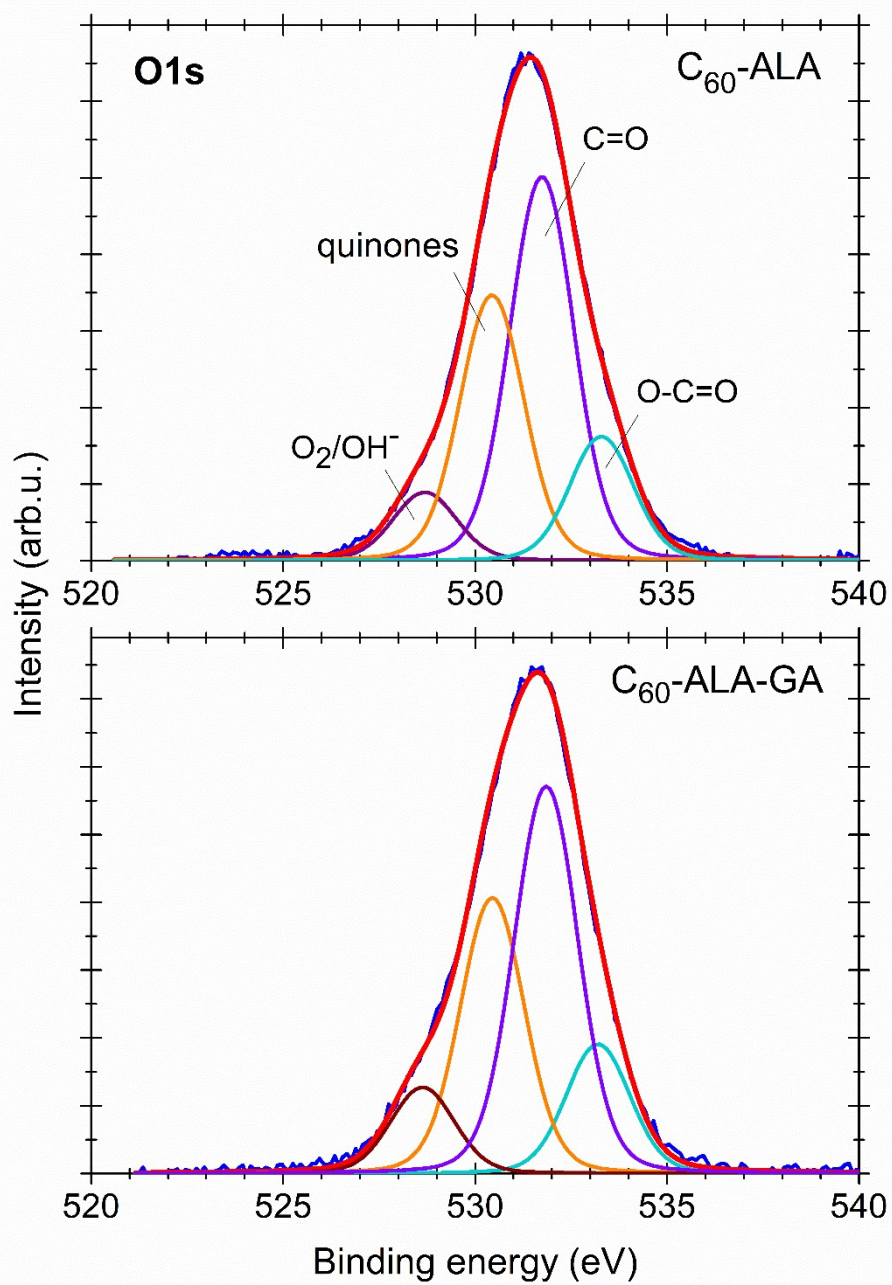


Figure S14

A high-resolution photoemission spectra of oxygen (O1s) measured for selected fullerene nanomaterials C₆₀-ALA and C₆₀-ALA-GA.

Compound	C (mg/mL)	MCF 7 (mg/mL)	A549 (mg/mL)	HCT116 (mg/mL)
CONTROL	0	0.37	0.41	0.78
5-ALA	1	0.67	0.47	1
C ₆₀ -ALA	1	0.71	0.66	1.34
C ₆₀ -ALA-GA	1	1.52	1.6	3.38

Table S2

Quantitative HPLC of protoporphyrin IX detected in the cellular lysates and measured at 400 nm after the treatment of cells with 5-ALA or fullerene nanomaterials. The MCF7, A549 and HCT116 cancer cell lines were used in this study. The final results were normalized to number of substrate mmols.

	C₆₀-ALA				C₆₀-ALA-GA			
Element	Atomic concentration [%]	Chemical states	Binding energy [eV]	Contributions of lines [%]	Atomic concentration [at %]	Chemical states	Binding energy [eV]	Contributions of lines [%]
C1s	64.3	C-Si	281.53	3.7	69.9	C-Si	281.54	3.7
		C=C	283.23	17.7		C=C	283.21	17.8
		C-H, C-C	284.89	55.4		C-H, C-C	284.90	51.1
		C-O, C-N, -C-OH	286.41	11.4		C-O, C-N, -C-OH	286.36	17.7
		O=C-OH, C=O	288.27	11.8		O=C-OH, C=O	288.28	9.8
O1s	24.0	O ₂ /OH ⁻	528.69	8.1	17.7	O ₂ /OH ⁻	528.68	10.5
		quinones	530.46	31.5		quinones	530.49	32.1
		C=O	531.89	45.6		C=O	531.93	44.7
		O-C=O	533.26	14.8		O-C=O	533.28	12.7
N1s	7.8	C-N, N-(C=O)-	398.43	31.4	8.1	C-N, N-(C=O)-	398.35	34.4
		pyridinic N	399.79	60.9		pyridinic N	399.76	54.4
Na1s	3.5	-	-	-	1.1	-	-	-
Si2p	0.2	-	-	-	1.7	-	-	-
K2p	-	-	-	-	1.1	-	-	-
F1s	-	-	-	-	0.2	-	-	-
Cl2p	0.1	-	-	-	0.1	-	-	-

S2p	0.1	-	-	-	0.1	-	-	-
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Table S3

Chemical composition and atomic concentrations for C₆₀-ALA and C₆₀-ALA-GA, determined from XPS measurements.

<i>Gene name</i>	<i>Sequence of primer</i>	
PEPT1	F	AGGCAACA ACTATGTCCGGG
	R	CACAGCATCGAAGATCGGGA
ABCG2	F	GCACAGGAAGTTTACGCACAG
	R	AAGGGGCTAGAAGAAGGGGG
FECH	F	GATGAATTGTCCCCAACAC
	R	GCTTCGTCCCACTTGATTA
HO-1	F	CATCCCCTACACACCAGCCA
	R	ATGTTGGGGAAGGTGAAGAAGG
GAPDH	F	GAGTCAACGGATTTGGTCGTA
	R	GCCCCACTTGATTTTGGAG

Table S4

The sequences of the primers used in RT-PCR experiments.

Compound	C [mg/mL]	C [mmol/L]	C_{max} 5-ALA
C ₆₀ -ALA	1	0.464	4.176
C ₆₀ -ALA-GA	1	0.157	1.413
5-ALA	1	5.97	5.97

Table S5

The conversion of concentration units for selected fullerene nanomaterials.