

# A highly water-soluble C<sub>60</sub>-NVP copolymer: a potential material for photodynamic therapy

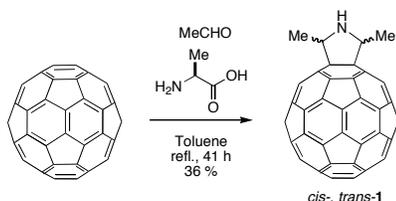
## Supporting Information

Yuko Iwamoto and Yoko Yamakoshi\*

Department of Chemistry and Biochemistry, University of California at Santa Barbara  
CA93106-9510 USA

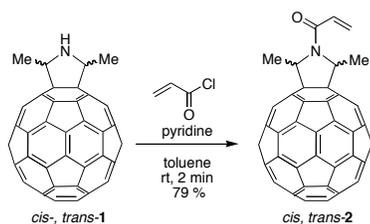
## Synthesis of co-polymer of C<sub>60</sub> and N-vinylpyrrolidone

**General.** Melting points were measured with MELTEMP (Laboratory Devices, Holliston, MA) and are uncorrected. NMR spectra were recorded on Varian Unity 400 spectrometer (Varian Inc., CA). Mass Spectrometry was performed by VG70 doublefocusing mass spectrometer (VG Analytical, Manchester, UK). UV and IR spectra were recorded on JASCO V-530 and JASCO FT/IR-430 (JASCO, Tokyo, JPN), respectively. HPLC analysis was carried out by JASCO PU-1580 intelligent HPLC pump, JASCO UV-1570 intelligent UV/VIS detector and ChromPass Chromatography Data System (JASCO, Tokyo, JPN). All the solvents were purchased from EMD Chemicals Inc., NJ, and dried by solvent system (Innovative Technology Inc., FL) or distilled if needed. All the reagents were purchased from corresponding suppliers and purified as described when needed. Column chromatography, preparative TLC and analytical TLC were performed on silica gel (Merck Silicagel 60 F<sub>254</sub> (Art 5715) 0.25mm, EM Science silica gel 60 PF<sub>254</sub> and E. Merck Silica gel 60 (230 – 400 mesh), respectively).



**Dimethylfulleropyrrolidine, 1.**<sup>1</sup> To a solution of C<sub>60</sub> (99.5 %, Material Technology Research Ltd., OH, 144 mg, 0.20 mmol) in toluene (100 mL), L-alanine (Tokyo Chemical Industry Co., Ltd., JPN, 36 mg, 0.40 mmol) and acetaldehyde (Alfa Aesar, MA, distilled, 44 mg, 1.0 mmol) were added and refluxed for 41 hours under nitrogen atmosphere. The reaction process was monitored by TLC (SiO<sub>2</sub>, toluene-EtOAc (10 : 1), R<sub>f</sub> of product: 0.19) and HPLC (column: Senshu Pak Silica-1151-N (Φ4.6x150 mm), solvent: toluene-EtOAc (10 : 1), flow rate: 1.0 mL/min, detection wavelength: 390 nm, injection: 10 μL, retention times: 1.68 min (C<sub>60</sub>), 11.48 min (*cis-1*), 12.16 min (*trans-1*)). The reaction mixture was separated by silica gel column chromatography (SiO<sub>2</sub>: 150 g, developed with hexane, hexane-toluene, hexane-toluene-EtOAc, eluted with hexane-toluene-EtOAc (5 : 5 : 1)) to give brown solid *cis* and *trans-1* (57 mg, 0.072 mmol,  $y = 36\%$ , recovered C<sub>60</sub> = 66 mg (0.092 mmol) and conversion  $y = 67\%$ ). The ratio of *cis* and *trans* isomers were calculated as 77 : 23 by integration of <sup>1</sup>H-NMR spectra. TLC: R<sub>f</sub> 0.55 (toluene-EtOAc (2 : 1)); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): *cis-1* δ 5.12 (q,  $J=6.78$ , 2H), 2.07 (d,  $J=6.87$ , 6H), *trans-1* δ 4.85 (q,  $J=6.59$ , 2H), 2.05 (d,  $J=6.69$ , 6H); FAB-MS (matrix: NBA)  $m/z$  792 [M+1]<sup>+</sup>.

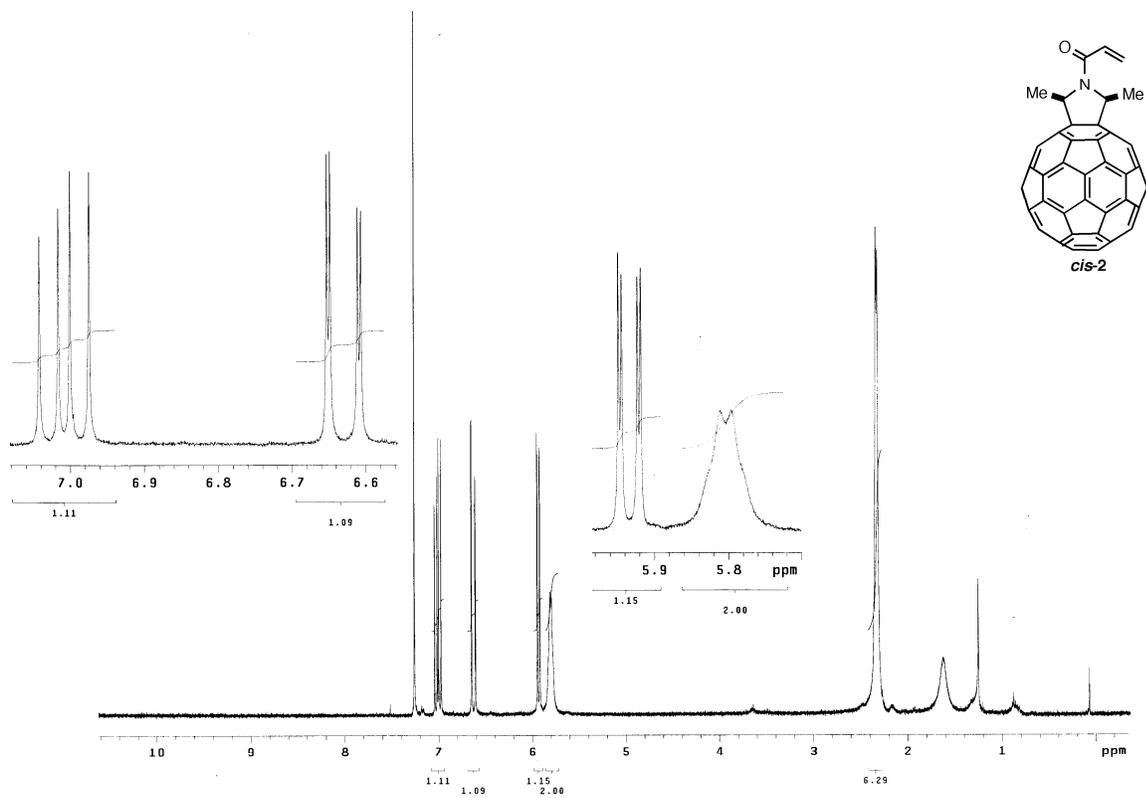
<sup>11</sup> Tan, X.; Schuster, D. I.; Wilson, S. R. *Tetrahedron Lett.* **1998**, 39, 4187-4190.



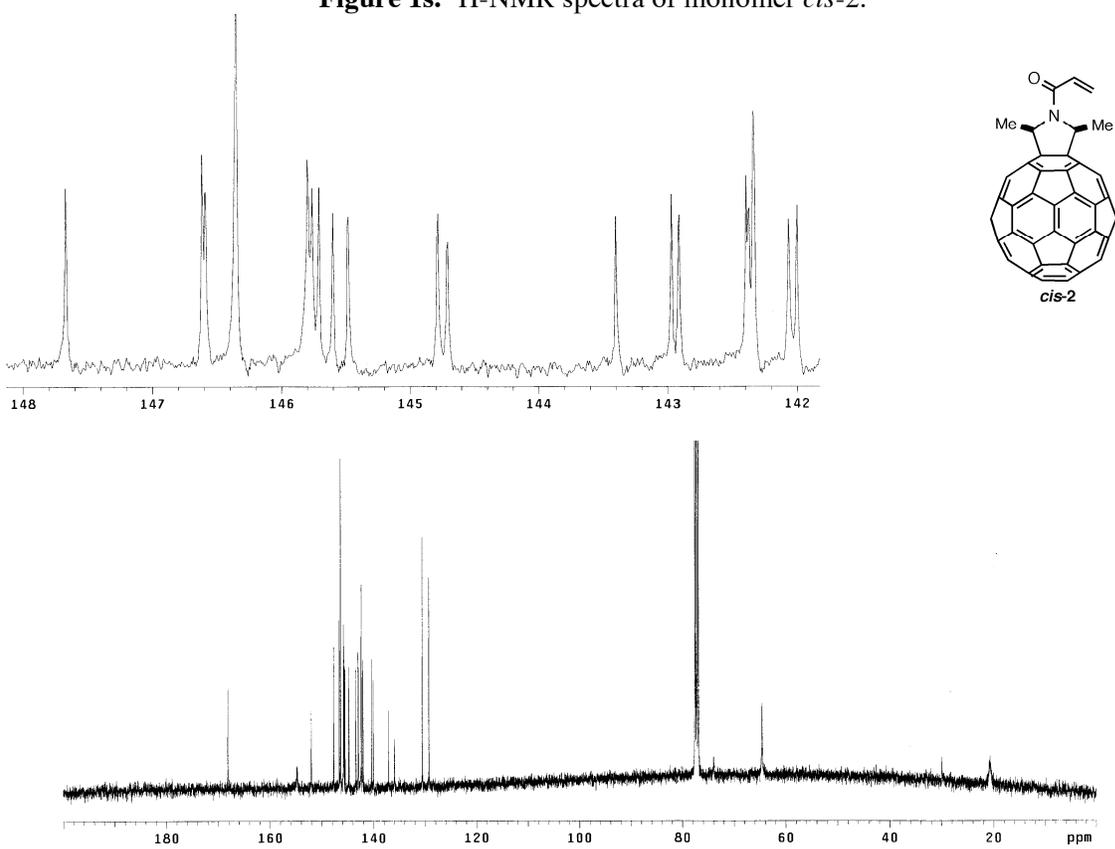
***N*-Carboxyvinylfulleropyrrolidine, 2.** To a solution of *cis* and *trans-1* (34 mg, 0.043 mmol) in toluene (20 mL), acryloyl chloride (TCI, 40  $\mu$ L, 45 mg, 0.46 mmol) and pyridine (Alfa Aesar, distilled, 2 mL) were added and stirred for 2 min under N<sub>2</sub> atmosphere. The reaction progress was monitored by TLC (SiO<sub>2</sub>, toluene – EtOAc (10 : 1), Rf: *cis-, trans-1*: 0.19, *cis-2*: 0.44, *trans-2*: 0.37). The reaction mixture was separated by silica gel column chromatography (SiO<sub>2</sub>: 50g, developed with hexane, toluene-EtOAc, eluted with toluene-EtOAc (30 : 1)) to give a brown solid (*cis-* and *trans-2*, 30.3 mg), which was purified further by preparative TLC (toluene-EtOAc(10:1)) to give *cis-2* (20.4 mg, 0.024 mmol) and *trans-2* (8.2 mg, 0.0097 mmol) (total 0.034 mmol, yield = 79 %).

*cis-2*: mp >260 °C; IR (neat) 2969 cm<sup>-1</sup>, 2925, 2848, 1649, 1609, 1415, 1376, 1303, 1277, 1213, 1185, 1099, 972; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.01 (dd,  $J = 10.29, 16.74$  Hz, 1H, vinyl), 6.63 (dd,  $J = 16.74, 1.84$  Hz, 1H, vinyl), 5.93 (dd,  $J = 10.29, 1.84$  Hz, 1H, vinyl), 5.80 (br.q,  $J = 5.99$ , 2H, CH), 2.32 (d,  $J = 5.99$ , 6H, CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  168.1 (C=O), 154.8, 152.0, 147.7, 146.6, 146.6, 146.4 (3 peaks overlap), 145.8 (2 peaks overlap), 145.8, 145.7, 145.6, 145.5, 144.8, 144.7, 143.4, 143.0, 142.9, 142.4, 142.4, 142.3 (2 peaks overlap), 142.1, 142.0, 140.4, 140.0, 137.0, 135.9, 130.6 (vinyl), 129.2 (vinyl), 74.0 (sp<sup>3</sup> quaternary), 64.6 (CH), 20.2 (CH<sub>3</sub>); MS (FAB, NBA)  $m/z$  846 ([M+H]<sup>+</sup>); HR-MS (ESI) 868.0772 obs., 868.0732 calcd. for C<sub>67</sub>H<sub>11</sub>NONa.

*trans-2*: mp >260°C; IR (neat) 3007 cm<sup>-1</sup>, 2967, 2925, 2851, 1666, 1614, 1407, 1378, 1338, 1264, 1227, 1186, 1123, 972; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  6.97 (dd,  $J = 10.3, 16.7$  Hz, 1H, vinyl), 6.74 (dd,  $J = 16.7, 2.00$  Hz, 1H, vinyl), 6.20 (q,  $J = 7.06, 2$  Hz, CH), 6.00 (dd,  $J = 10.3, 2.00$  Hz, 1H, vinyl), 2.28 (d,  $J = 7.06, 6$  Hz, CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  164.1 (C=O), 155.8, 152.1, 147.7, 146.6, 146.5, 146.4, 146.3, 146.2, 145.8, 145.6 (2 peaks overlap), 145.6, 145.5, 144.9, 144.8, 144.6, 143.5, 142.9, 142.9, 142.5, 142.4, 142.3 (2 peaks overlap), 142.2, 141.8, 140.4, 140.2, 137.6, 134.5, 130.2 (vinyl), 127.8 (vinyl), 73.6 (sp<sup>3</sup> quaternary), 64.1 (CH), 24.7 (CH<sub>3</sub>); MS (FAB, NBA)  $m/z$  846 ([M+H]<sup>+</sup>); HR-MS (ESI) 868.0730 obs., 868.0732 calcd. for C<sub>67</sub>H<sub>11</sub>NONa.



**Figure 1s.**  $^1\text{H-NMR}$  spectra of monomer *cis-2*.



**Figure 2s.**  $^{13}\text{C-NMR}$  spectra of monomer *cis-2*.

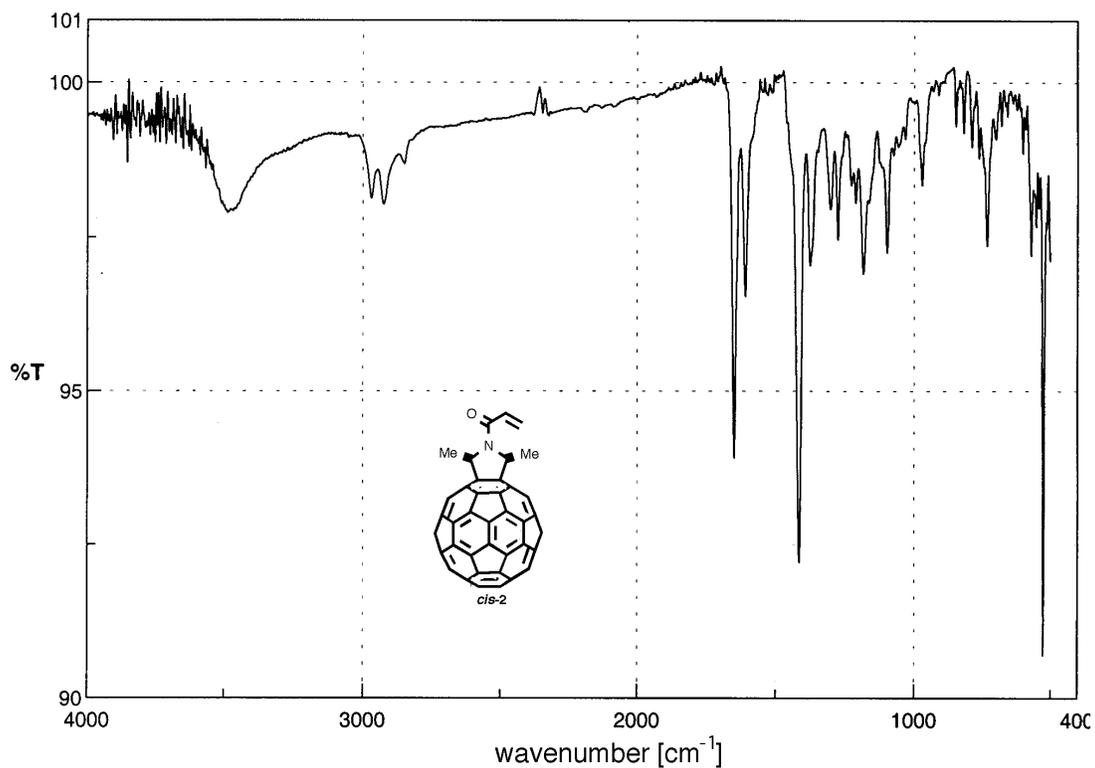


Figure 3s. IR spectra of monomer *cis-2*.

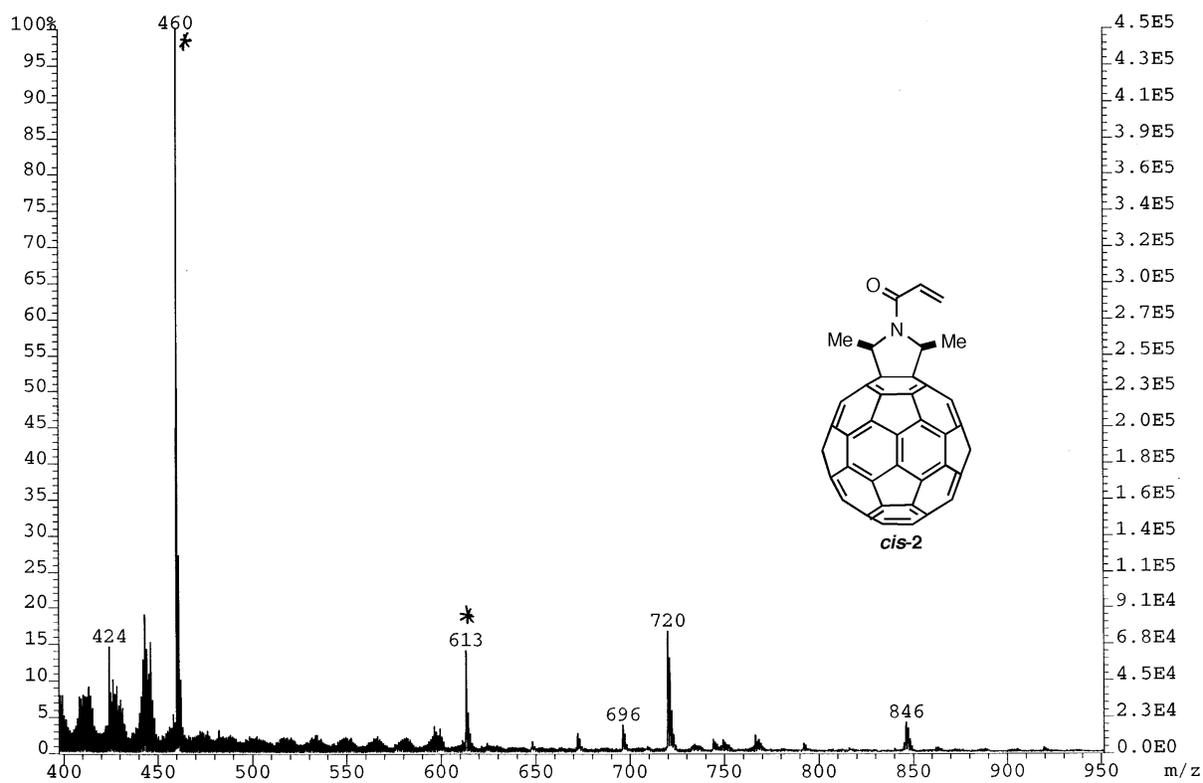
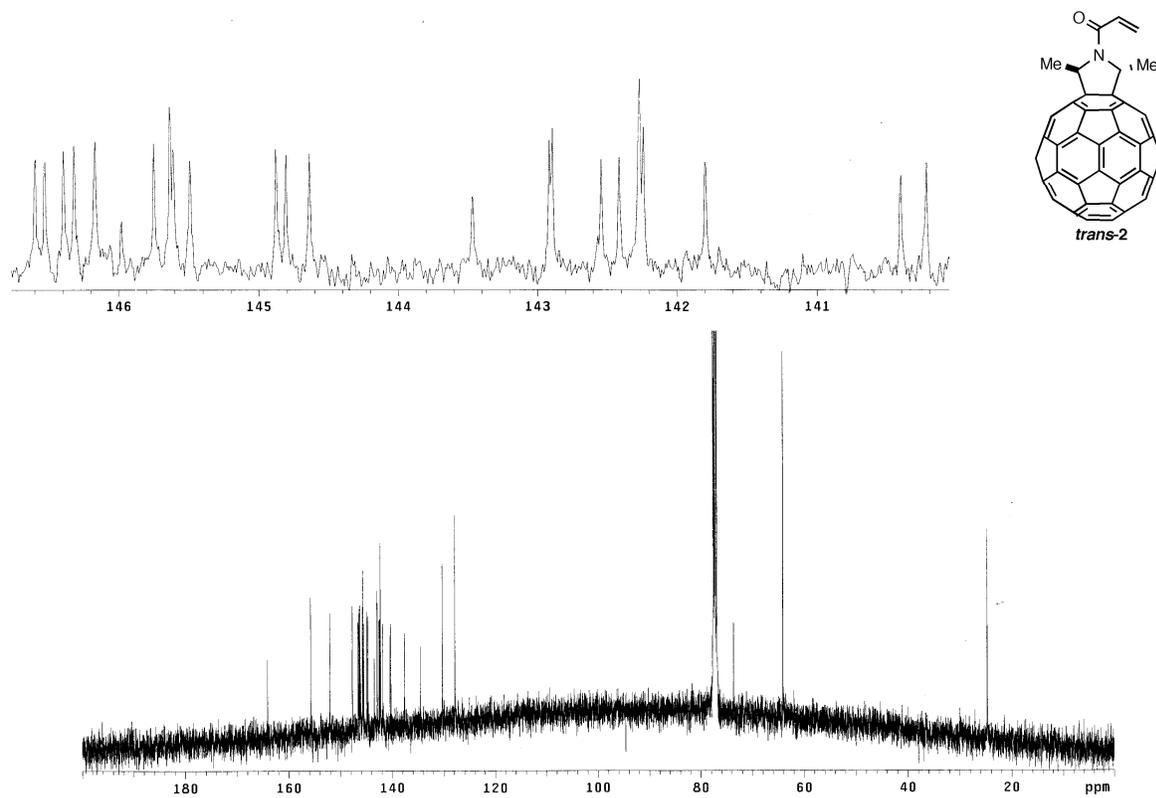
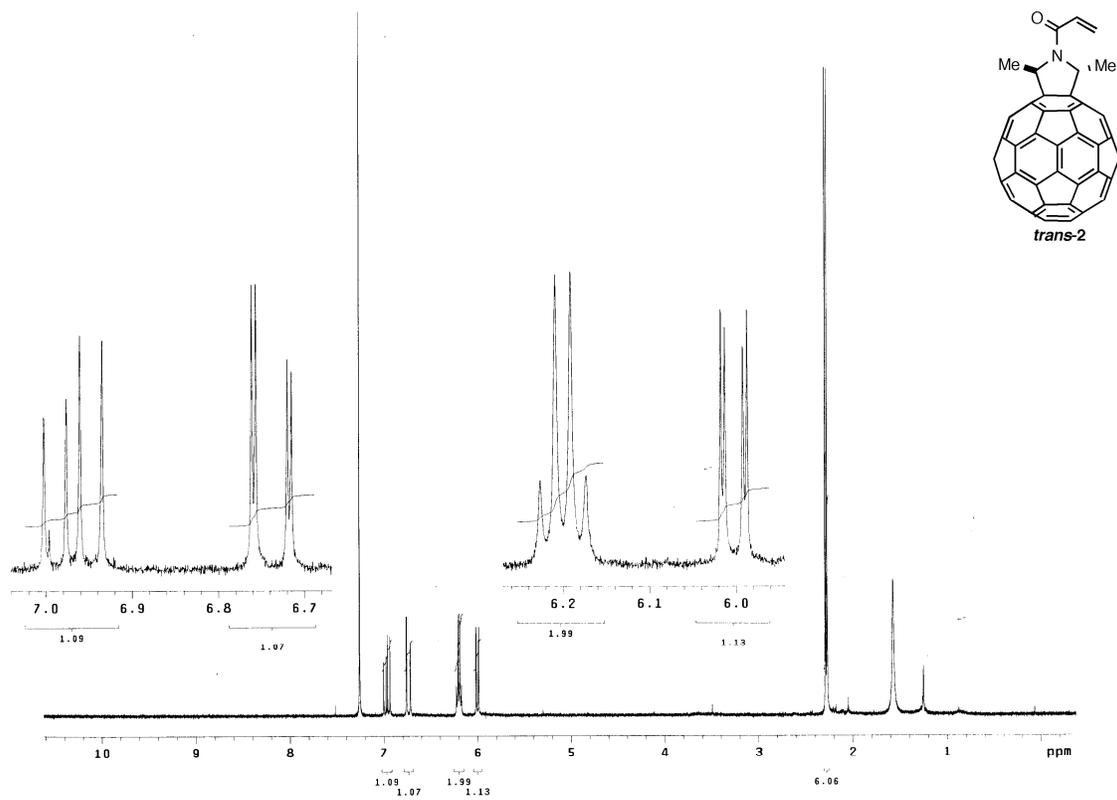


Figure 4s. MS spectra of monomer *cis-2*.



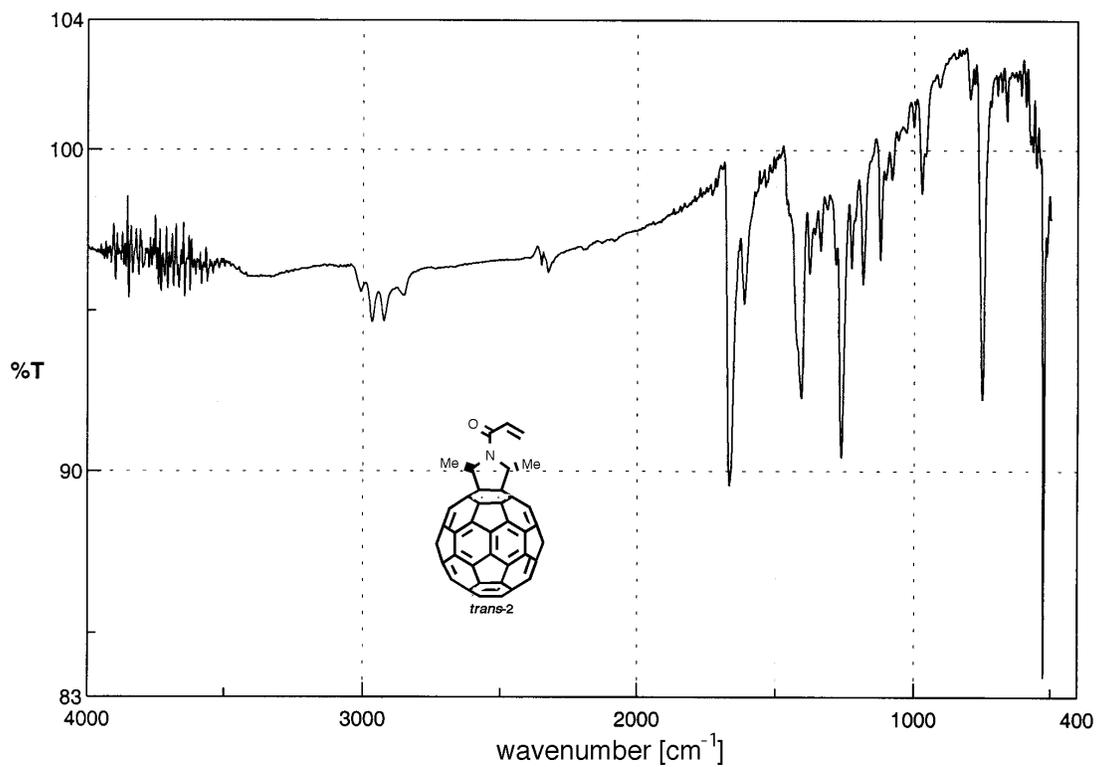


Figure 7s. IR spectra of monomer *trans*-2.

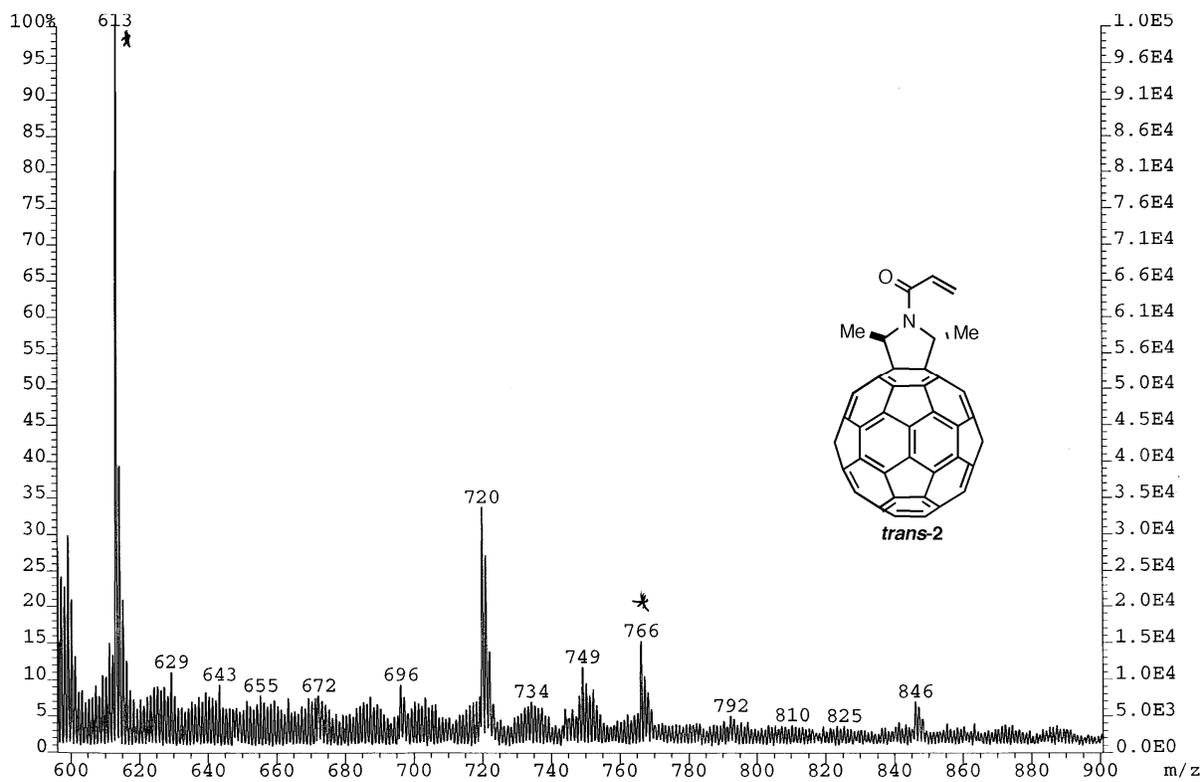
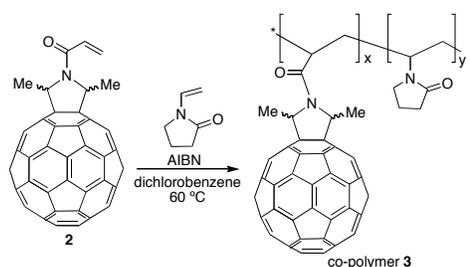


Figure 8s. MS spectra of monomer *trans*-2.



**General procedure for the preparation of polymers 3a-g.** To a solution of *cis*- and *trans*- **2** in dichlorobenzene (TCI), *N*-vinylpyrrolidone (TCI) and azoisobutyronitrile (AIBN, Aldrich) were added. Reaction mixture was degassed by freeze-pump-thaw (5 cycles) and stirred under nitrogen atmosphere at 60 °C. Normally, the reaction mixture became solid during the reaction. The reaction mixture was reconstituted with a small amount of  $\text{CHCl}_3$  and subsequently 20 mL of  $\text{Et}_2\text{O}$  was added to precipitate (two times) a brown substance, which was dried in a vacuum oven (60 °C) to give a brown powder.

**Table Is.** Reaction conditions and yields of copolymer preparations.

co-polymer	feed ratio of <b>2</b> and NVP	feed amount of reagents			solvent [mL]	time [hours]	polymer obtained [mg]	Yield [%]
		<b>2</b> [mg] (mmol)	NVP [mg] (mmol)	AIBN [mg] (mmol)				
<b>3a</b>	1:50	7.4 (0.0087)	49 (0.44)	14 (0.086)	0.06	41	24	43
<b>3b</b>	1:75	8.5 (0.010)	83 (0.75)	25 (0.15)	0.10	72	49	53
<b>3c</b>	1:100	8.5 (0.010)	111 (1.0)	33 (0.20)	0.13	46	79	66
<b>3d</b>	1:150	17.3 (0.020)	333 (3.0)	99 (0.60)	0.40	40	295	84
<b>3e</b>	1:200	17.2 (0.020)	447 (4.0)	131 (0.80)	0.53	24	447	96
<b>3f</b>	1:250	9.0 (0.011)	296 (2.7)	88 (0.53)	0.34	24	292	96
<b>3g</b>	1:300	6.4 (0.0076)	252 (2.3)	75 (0.46)	0.3	24	245	95

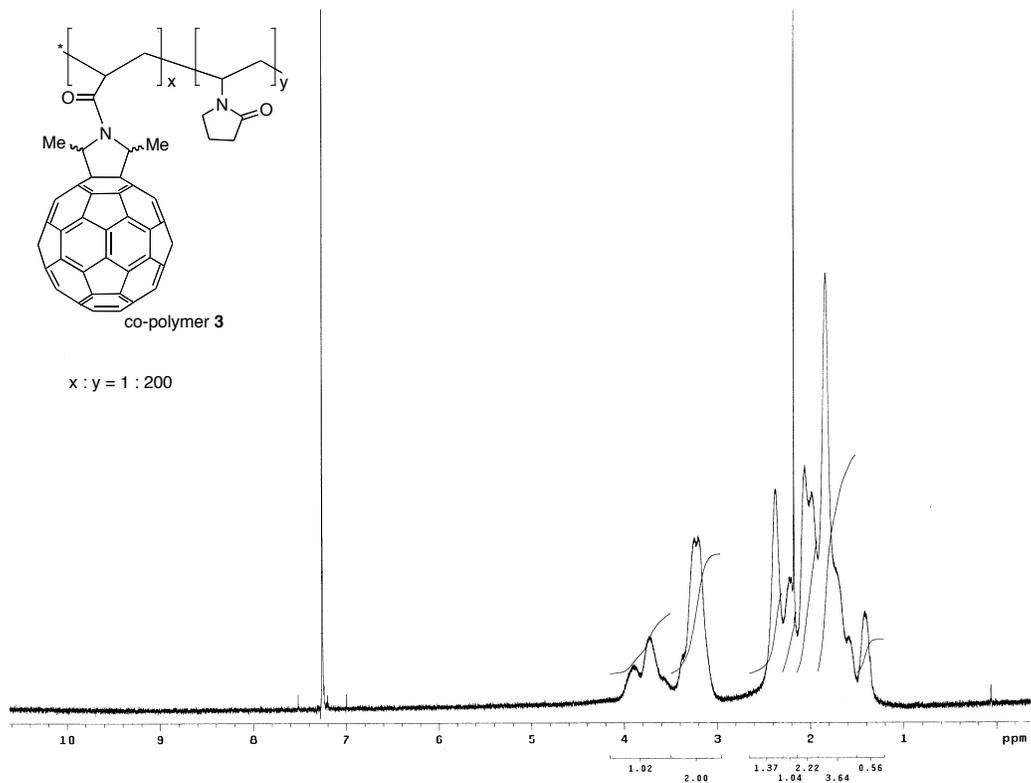


Figure 9s.  $^1\text{H-NMR}$  spectra of copolymer 3e.

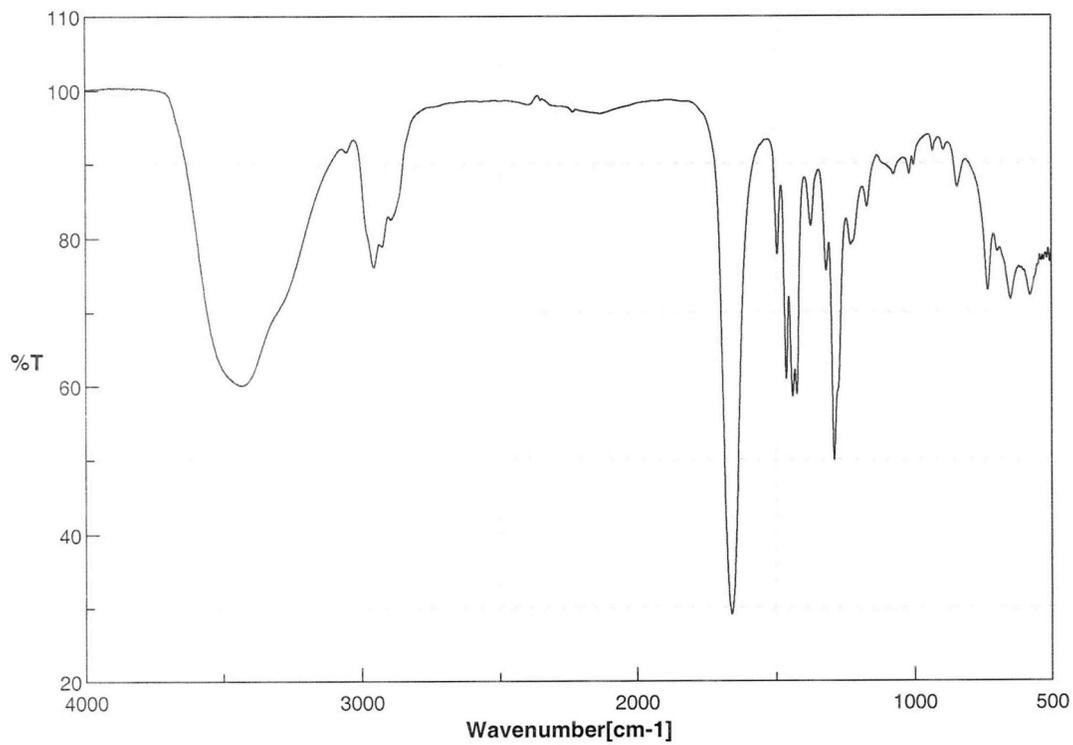


Figure 10s. IR spectra of copolymer 3e.

### GPC analysis of C<sub>60</sub>-NVP co-polymers

GPC analysis was performed by Waters 2690 separation module and Waters 2410 RI detector (Waters Co., MA) equipped with a Styragel column ( $\Phi$ 7.8x300 mm Waters, Co. MA). An aliquot of each C<sub>60</sub>-NVP copolymer (6 mg), 2.0 mL of DMF with 0.1% of LiBr was added and was analyzed after filtration (Acrodisk®, 0.45  $\mu$ m, PTFE CR13 pall). Elution was carried out by DMF with 0.1% of LiBr. The calibration was carried out with standard polystyrenes with MW 947 Da – 188 kDa.

### DSC analysis of C<sub>60</sub>-NVP co-polymers

Differential scanning calorimetry (DSC) analysis was carried out by DSC 2920 (TA instrument, New Castle, DE). An aliquot of each C<sub>60</sub>-NVP copolymer (3-4 mg) was measured and analyzed. Temperature was raised from 0 °C to 210 °C by the rate of 10 °C / min, then cooled to -30 °C by the rate of 5 °C / min to determine glass transition temperature ( $T_g$ ) of each copolymer.

### DLS analysis of C<sub>60</sub>-NVP co-polymers

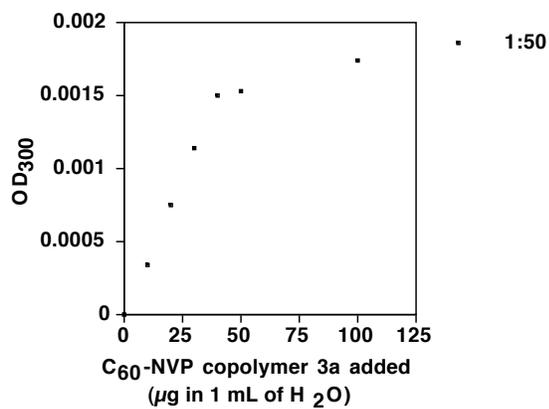
Dynamic light scattering (DLS) analysis was carried out by BI-9000AI digital correlator, BI-APD Avalanche photodiode (Brookhaven Instruments Ltd., NY) equipped with 633 10 mW HeNe laser with power module. An aliquot of each C<sub>60</sub>-NVP copolymer was measured and water, DMSO or DMF was added to prepare 1.25 mg/mL solution. After filtration (Nalgene syringe filter 0.45  $\mu$ m, No.190-2545 for aqueous solution and Alltech Polypure™ syringe filter 0.45  $\mu$ m for DMSO and DMF solution), the solution was analyzed.

**Table II.** Characterization of C<sub>60</sub>-NVP copolymers.

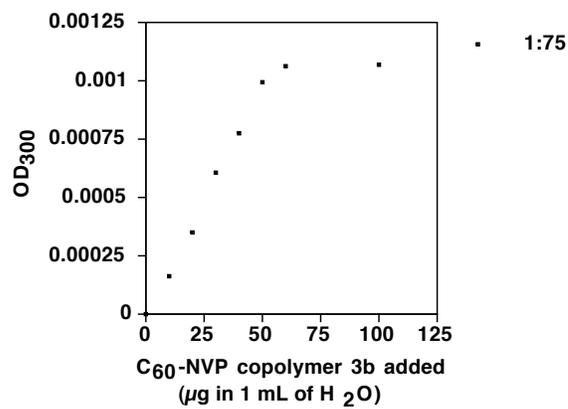
Ratio of feed C <sub>60</sub> and NVP	1:50	1:75	1:100	1:150	1:200	1:250	1:300
Mn by GPC [kDa]	41	41	30	43	40	46	51
Grass transition temperature by DSC [°C]	151	160	149	163	173	168	169
Diameter of polymer granule by DLS [nm]							
in H <sub>2</sub> O	not soluble	not soluble	84.2	23.9	19.5	18.1	19.2
in DMSO	89.7	13.5	17.4	15.2	15.5	10.0	8.6
in DMF	85.8	18.2	22.4	18.0	20.3	21.8	12.3

## Measurement of solubility of C<sub>60</sub>-NVP co-polymers

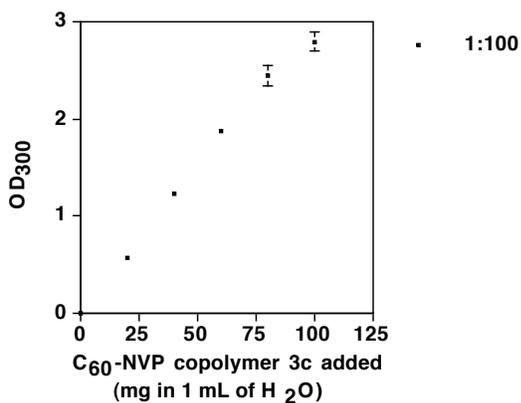
To an aliquot of C<sub>60</sub> polymer, DI water was added to make 1 mL solution and treated by sonication for 2 min. The solution was filtered through a cotton-plugged filter and subsequently a membrane filter (Millex®-GV, Millipore Co., MA) to remove the insoluble solid. The filtrate was diluted with DI water (1/100) to measure the absorbance at 300 nm. All the measurements were carried out for three times.



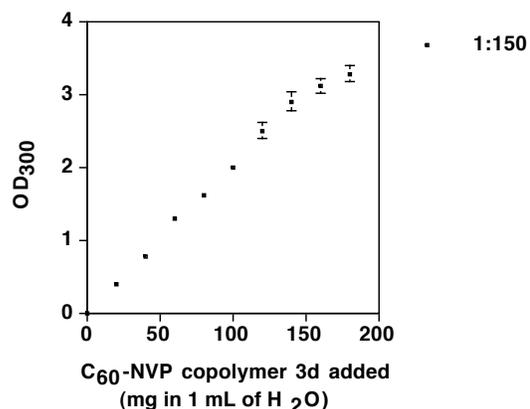
**Figure 11s-a.** Solubility curves of C<sub>60</sub>-NVP copolymer (1:50) in water.



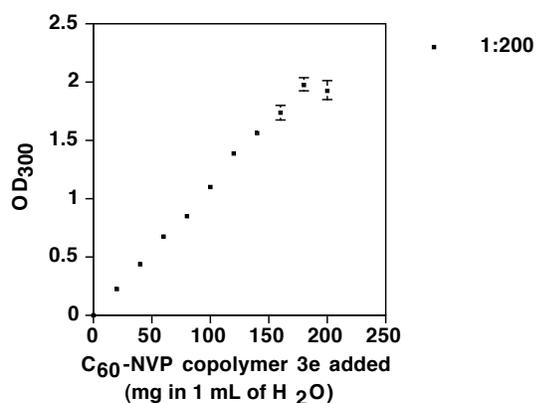
**Figure 11s-b.** Solubility curves of C<sub>60</sub>-NVP copolymer (1:75) in water.



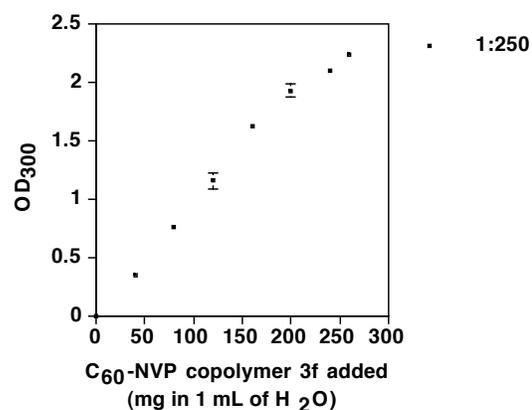
**Figure 11s-c.** Solubility curves of C<sub>60</sub>-NVP copolymer (1:100) in water.



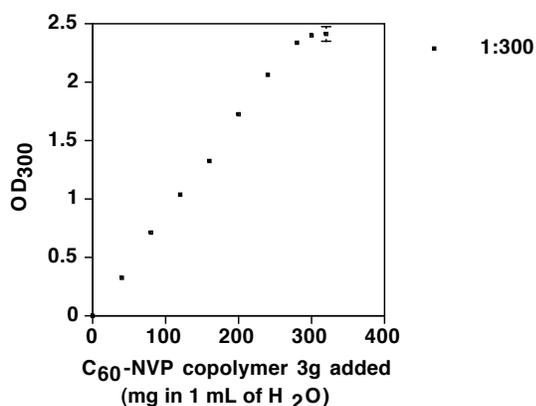
**Figure 11s-d.** Solubility curves of C<sub>60</sub>-NVP copolymer (1:150) in water.



**Figure 11s-e.** Solubility curves of C<sub>60</sub>-NVP copolymer (1:200) in water.



**Figure 11s-f.** Solubility curves of C<sub>60</sub>-NVP copolymer (1:250) in water.



**Figure 11s-g.** Solubility curves of C<sub>60</sub>-NVP copolymer (1:300) in water.

**Figure 9s.** Solubility curves of C<sub>60</sub>-NVP polymer in water.

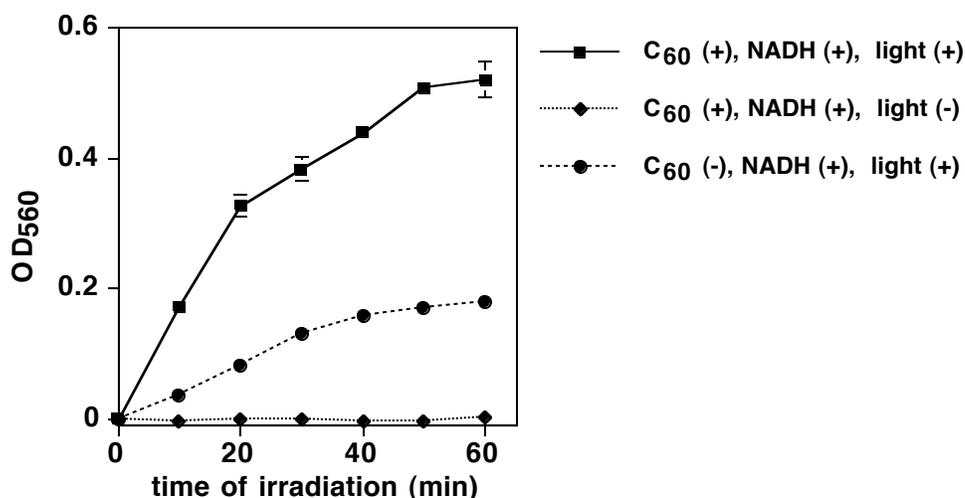
**Table IIIs.** Solubility of C<sub>60</sub>-NVP copolymers in water.

Ratio of feed C <sub>60</sub> and NVP	1:50	1:75	1:100	1:150	1:200	1:250	1:300
Solubility (mg/mL)	0.04	0.05	80	120	180	200	240
Concentration of C <sub>60</sub> (mM calculated as monomer)	0.0063	0.0055	6.7	6.9	7.8	7.0	7.0
Concentration of PVP (%w/v)	0.0035	0.0045	7.4	11	17	19	23

## Detection of superoxide radical anion from C<sub>60</sub>-NVP co-polymer by NBT method

The generation of O<sub>2</sub><sup>•-</sup> from C<sub>60</sub>-NVP co-polymer (1 : 200) was detected by NBT (nitro blue tetrazolium) method as reported by Nagano and co-workers.<sup>2</sup> Assay was performed in 50 mM phosphate buffer (pH 6.5) in the presence of NBT 0.24 mM, EDTA 0.1 mM, NADH 10 mM.

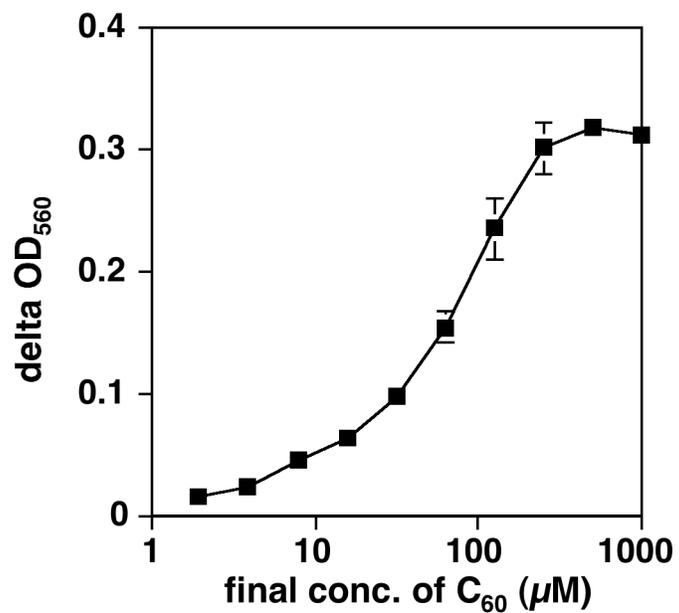
For the time course measurement, solution with C<sub>60</sub>-NVP copolymer 11.5 mg/mL (estimated concentration of C<sub>60</sub> as monomer: 0.5 mM) was incubated under or without photoirradiation using two 200-W photoreflector lamps with 12-13 cm of distance through glass (35,000-37,000 lux) in the ice bath (0-5 °C). As a reference, 1.1 % of PVP was used. An aliquot (200 μL) of each reactant was subsequently diluted with 50 mM phosphate buffer (1800 μL) before measuring the absorbance at 560 nm.



**Figure 12s.** Time course of photoinduced O<sub>2</sub><sup>•-</sup> generation by C<sub>60</sub>-NVP copolymer in 50 mM phosphate buffer (pH 6.5). Irradiation: two 200-W photoreflector lamps (35,000 – 37,000 lux) at 0-5°C. In the presence of C<sub>60</sub> copolymer (11.5 mg/mL, 50 mM of C<sub>60</sub>) and NADH (10 mM), under visible light irradiation (■); in the presence of C<sub>60</sub> copolymer and NADH, without light irradiation (◆); without C<sub>60</sub> copolymer, with NADH, under light irradiation (●).

For the dose-dependent experiment, solutions with corresponding concentrations of C<sub>60</sub>-NVP copolymer was prepared and incubated under same conditions of photoirradiation for 30 min.

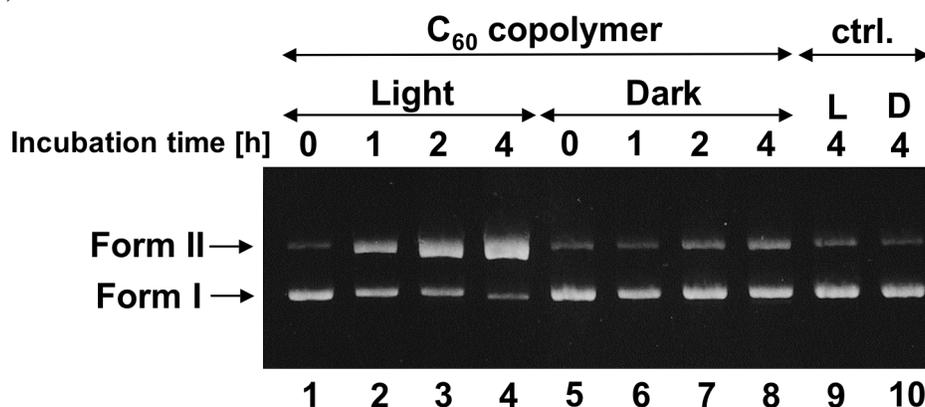
<sup>2</sup> Umezawa, N.; Arakane, K.; Kyu, A.; Mashiko, S.; Hirobe, M.; Nagano, T. *Arch. Biochem. Biophys.* **1997**, *342*, 275-281.



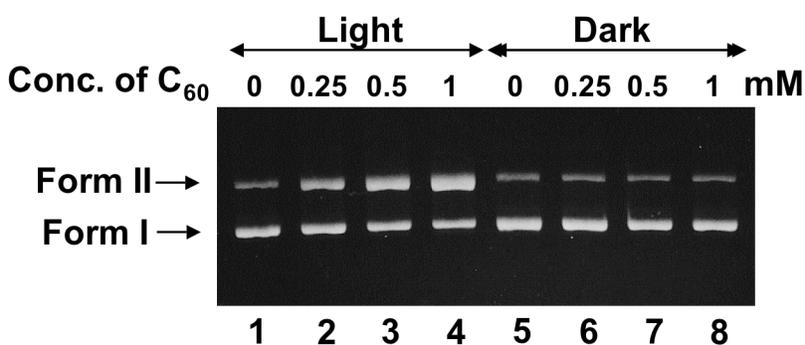
**Figure 13s.** Dose-dependent effect of C<sub>60</sub> on O<sub>2</sub>•<sup>-</sup> generation by C<sub>60</sub>-NVP copolymer in 50 mM phosphate buffer (pH 6.5). Irradiation: : two 200-W photoreflector lamps (35,000 – 37,000 lux) at 0-5°C for 30 min.

## DNA-cleaving test of C<sub>60</sub>-NVP copolymers

A super coiled plasmid DNA (pBR322, 1000  $\mu\text{g/mL}$ , New England Biolabs Inc., MA) was diluted with 1 M TDC buffer (pH 8.0) to 100  $\mu\text{g/mL}$ . The DNA solution, C<sub>60</sub>-NVP copolymer solutions and other reagent solutions (reductant) were mixed well in TDC buffer (Tris-HCl buffer with KCl and MgCl<sub>2</sub>) and subjected to photoirradiation with a 200-W photoreflector lamp at a distance of 12-13 cm (60,000-63,000 lux) at 37 °C. After irradiation, an aliquot (20  $\mu\text{L}$ ) of the irradiated solution was mixed with bromophenol blue-glycerol solution (5  $\mu\text{L}$ ) and subjected to the agarose gel electrophoresis (1 % agarose in Timiza base EDTA (TBE) buffer, 100 V, 70 min). The gel was stained with ethidium bromide and photographed on the transilluminator for analysis of the ratio of Form I (supercoiled) and Form II (nicked).



**Figure 14s.** Photoinduced DNA cleavage by water-soluble C<sub>60</sub>-NVP copolymer (time dependency of visible light irradiation). The pBR322 supercoiled plasmid was incubated with 23 mg/mL (1 mM of C<sub>60</sub> estimated as monomer) of C<sub>60</sub>-NVP copolymer (1 : 200) in TDC buffer in the presence of 10 mM of NADH at 37 °C for 0, 1, 2 and 4 hours under irradiation with a 200-W photoreflector lamp. Lanes 1-8, in the presence of C<sub>60</sub> copolymer; lanes 9, 10, without C<sub>60</sub> copolymer; lanes 1-4 and 9, incubated under visible light irradiation; lanes 5-8 and 10, incubated without light irradiation; lanes 1 and 5, incubated for 0 min; lanes 2 and 6, 1 h; lanes 3 and 7, 2h; lanes 4 and 8-10, 4 h.



**Figure 15s.** Photoinduced DNA cleavage by water-soluble C<sub>60</sub>-NVP copolymer (concentration dependency of copolymer). The pBR322 supercoiled plasmid was incubated with 0, 5.75, 11.5 and 23 mg/mL of C<sub>60</sub>-NVP copolymer (1 : 200) (0, 0.25, 0.5 and 1 mM of C<sub>60</sub> estimated) in TDC buffer at 37 °C for 2 hours under irradiation with two 200-W photoreflector lamps. Lanes 1-8, in the presence of C<sub>60</sub> copolymer; lanes 9, 10, without C<sub>60</sub> copolymer; lanes 1-4 and 9, incubated under visible light irradiation; lanes 5-8 and 10, incubated without light irradiation; lanes 1 and 5, in the absence of C<sub>60</sub> copolymer; lanes 2 and 6, 5.75 mg/mL of C<sub>60</sub> copolymer (C<sub>60</sub> 0.25 mM); lanes 3 and 7, 11.5 mg/mL (0.5 mM); lanes 4 and 8, 23 mg/mL (1 mM).