

## Supplementary Information

### **Light-Driven Nitrile Imine-Mediated Tetrazole-Ene Cycloaddition as a Versatile Platform for Fullerene Conjugation**

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## Chemicals

Fullerene (99.5%, catalog number: 379646), 4-(dimethylamino)pyridine (DMAP, 99%), pyridine and dry 1,2-dichlorobenzene were purchased from Sigma-Aldrich. *N,N'*-dicyclohexylcarbodiimide (DCC, 99%) and 4-formyl benzoic acid (96%) were purchased from Acros. *p*-Toluene sulfonyl hydrazide (98%) and sodium nitrite (98%) were purchased from Alfa Aesar. Poly(ethylene glycol) methyl ether (MeO-PEG, average  $M_n \sim 2000$  g/mol) was purchased from Rapp Polymere. *t*-Butylacrylate (99%) was purchased from Alfa Aesar and was passed through a column of basic alumina to remove inhibitor. 2,2'-Azobis(isobutyronitrile) (AIBN) was purchased from VWR and recrystallized twice from methanol. Acetone (p.a., Fisher), tetrahydrofuran (THF, p.a., VWR), and toluene (p.a., VWR) were used for surface procedures.

## Instruments and Characterizations

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy was performed using Bruker AM 400 spectrometers at 400 and 101 MHz, respectively. A sample was dissolved in  $\text{CDCl}_3$ . The  $\delta$ -scale is referenced to the  $\text{CDCl}_3$  ( $\delta = 7.26$  and  $77.2$  ppm, respectively).

SEC/ESI-MS (Size-Exclusion Chromatography coupled to Electrospray Ionization-Mass Spectrometry) spectra were recorded on a LXQ mass spectrometer (ThermoFisher Scientific) equipped with an atmospheric pressure ionization source operating in the nebulizer-assisted electrospray mode. A constant spray voltage of 3.75 and 4.5 kV for molecular tetrazole and polymeric tetrazole, respectively, and a dimensionless sweep gas flow rate of 2 and a dimensionless sheath gas flow rate of 12 were applied. The capillary voltage and the tube lens offset voltage were set to 60 and 110 V, respectively. The capillary temperatures were 350 and 300 °C for molecular tetrazole and polymeric tetrazole, respectively. Spectra were measured in

negative and positive mode for molecular tetrazole and polymeric tetrazole, respectively. The LXQ was coupled to a Series 1200 HPLC system (Agilent) that consisted of a solvent degasser (G1322A), a binary pump (G1312A) and a high-performance autosampler (G1367B), followed by a thermostated column compartment (G1316A). Separation was performed on two mixed-bead SEC columns (Polymer Laboratories, Mesopore  $250 \times 4.6 \mu\text{m}$ , particle diameter  $3 \mu\text{m}$ ) with pre-column (Mesopore  $50 \times 4.6 \mu\text{m}$ ) operating at  $30^\circ\text{C}$ . THF at a flow rate of  $0.3 \text{ mL/min}$  was used as the eluent. The mass spectrometer was coupled to the column in parallel to an RI detector (G1362A with SS420xA/D) in a setup described previously.<sup>1</sup>  $0.27 \text{ mL/min}$  of the eluent was directed through the RI detector and  $30 \mu\text{L/min}$  infused into the electrospray source after postcolumn addition of a  $0.1 \text{ mM}$  solution of sodium iodide in methanol at  $20 \mu\text{L/min}$  by a micro flow HPLC syringe pump (Teledyne ISCO, Model 100DM). The polymer solutions ( $20 \mu\text{L}$ ) with a concentration of  $\sim 1.5 \text{ mg/mL}$  were injected into the HPLC system.

*Orbitrap ESI-MS* Mass spectrum was recorded on a Q Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an atmospheric pressure ionization source operating in the nebulizer assisted electrospray mode. The instrument was calibrated in the  $m/z$  range 150–2000 using a standard containing caffeine, Met-Arg-Phe-Ala acetate (MRFA) and a mixture of fluorinated phosphazenes (Ultramark 1621) (all from Aldrich). A constant spray voltage of  $2.5 \text{ kV}$  and a dimensionless sheath gas of 6 and a sweep gas flow rate of 2 were applied. The capillary voltage and the S-lens RF level were set to  $68.0 \text{ V}$  and  $320^\circ\text{C}$ , respectively.

*Size Exclusion Chromatography (SEC) measurements* were performed on a Polymer Laboratories (Varian) PL-SEC 50 Plus Integrated System, comprising an autosampler, a PLgel  $5 \text{ mm}$  bead-size guard column ( $50 \times 7.5 \text{ mm}$ ), one PLgel  $5 \text{ mm}$  Mixed E column ( $300 \times 7.5 \text{ mm}$ ), three PLgel  $5 \text{ mm}$  Mixed C columns ( $300 \times 7.5 \text{ mm}$ ) and a differential refractive index detector using THF as the

eluent at 35 °C with a flow rate of 1 mL min<sup>-1</sup>. The present SEC system was calibrated using linear poly(methyl methacrylate) standards ranging from 700 to 2 × 10<sup>6</sup> g mol<sup>-1</sup>. The resulting molar mass distributions were determined by universal calibration using Mark-Houwink parameters for polystyrene ( $K = 14.1 \cdot 10^{-5}$  dL g<sup>-1</sup>,  $\alpha = 0.7$ ).

*ToF-SIMS* (Time-of-Flight Secondary Ion Mass Spectrometry) was performed on a TOF.SIMS5 instrument (ION-TOF GmbH, Münster, Germany), equipped with a Bi cluster liquid metal primary ion source and a non-linear time of flight analyzer. The Bi source was operated in the “high current bunched” mode providing 0.9 ns Bi<sub>3</sub><sup>+</sup> ion pulses at 25 keV energy and a lateral resolution of approx. 4 μm. The short pulse length allowed for high mass resolution (for C<sub>60</sub><sup>+</sup> >7800  $m/\Delta m$ ) to analyze the complex mass spectra of the immobilized organic layers. Images larger than the maximum deflection range of the primary ion gun of 500 × 500 μm<sup>2</sup> were obtained using the manipulator stage scan mode with a pixel distance of 10 μm. Positive polarity spectra were calibrated on the C<sup>+</sup>, CH<sup>+</sup>, CH<sub>2</sub><sup>+</sup>, CH<sub>3</sub><sup>+</sup>, and the C<sub>60</sub><sup>+</sup> peak. Primary ion doses were kept below 10<sup>11</sup> ions/cm<sup>2</sup> (static SIMS limit).

## Synthesis

### *4-(2-phenyl-2H-tetrazol-5-yl)benzoic acid (1) and tetrazole-functionalized PEG (2)*

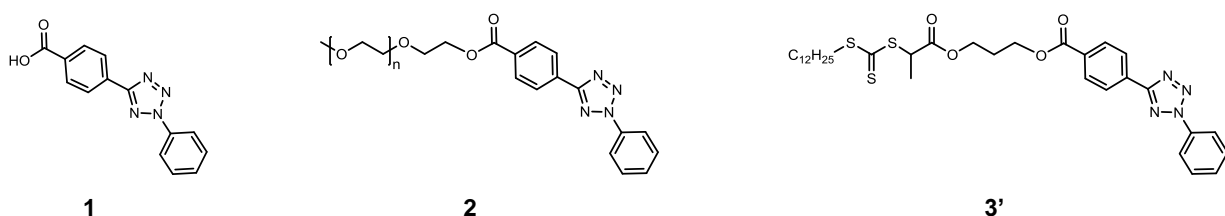
Carboxy-functionalized tetrazole, (4-(2-phenyl-2H-tetrazol-5-yl)benzoic acid, (**1**)<sup>2,3</sup> and tetrazole-functionalized PEG (**2**)<sup>3</sup> were synthesized according to previously published procedures. Briefly, 1.50 g 4-formyl benzoic acid and 1.86 g *p*-toluenesulfonyl hydrazide were dissolved in 25 mL ethanol, and the solution was refluxed for 4 h. The product was precipitated in water to obtain a white solid. 0.88 g aniline was cooled to 0°C in a solution of 2.5 mL concentrated hydrochloric acid, 8 mL water and 8 mL ethanol. A cooled solution of 0.65 g sodium nitrite in 4 mL water was

added dropwise. The *in-situ* generated diazonium salt was added dropwise to a solution of the white solid product in 50 mL pyridine. After the complete addition, the solution was stirred overnight at ambient temperature. The tetrazole **1** is precipitated in water to obtain the red solid **1**. 293 mg of **1**, 585 mg MeO-PEG, and 120 mg DCC were dissolved in a mixture of 10 mL dry DCM and 1 mL of DMF. 27 mg DMAP was added and the solution was stirred overnight. **2** was obtained by precipitation in diethyl ether.

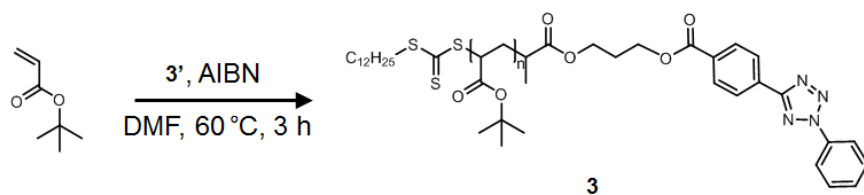
### *Tetrazole-functionalized PTBA (3)*

The tetrazole-functionalized RAFT agent (**3'**) was synthesized according to a previously published procedures.<sup>4</sup> A solution of AIBN (5.0 mg, 0.030 mmol, 0.20 eq.), the RAFT agent (100 mg, 0.15 mmol, 1.00 eq.) and *t*-butylacrylate (0.977 g, 7.62 mmol, 50 eq.) in 3.9 mL DMF was deoxygenated with four consecutive freeze-pump-thaw cycles. The reaction mixture was placed into a preheated oil bath at 60 °C for 3 h and subsequently quenched by cooling in an ice bath and exposure to oxygen. The product was isolated via dialysis in ethanol employing a SpectraPor3 membrane (MWCO = 500 Da). Subsequently, the reaction mixture was dried under vacuum to obtain 403 mg of a yellow solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.34 (d, J = 8.4 Hz, 2H, ArH), 8.22 (d, J = 7.2 Hz, 2H, ArH), 8.19 (d, J = 7.8 Hz, 2H, ArH), 7.60 (dd, J = 7.8, 6.6 Hz, 2H, ArH), 7.53 (t, J = 7.4 Hz, 1H, ArH), 4.64–4.73 (br, 1H, S–CH(CO)–CH<sub>2</sub>), 4.44 (t, J = 6.0 Hz, 2H, CH<sub>2</sub>–CH<sub>2</sub>–O), 4.18–4.32 (br, 2H, O–CH<sub>2</sub>–CH<sub>2</sub>), 3.32 (t, J = 7.4 Hz, 2H, CH<sub>2</sub>–CH<sub>2</sub>–S), 2.46 (br, 1H, CH<sub>2</sub>–CH(CH<sub>3</sub>)–CO), 2.23 (br, poly(*t*-butylacrylate), CH(CO)–CH<sub>2</sub>), 1.83 (br, poly(*t*-butylacrylate), CH(CO)–CH<sub>2</sub>), 1.67 (t, J = 7.4 Hz, 2H, CH<sub>2</sub>–CH<sub>2</sub>–S), 1.62 (br, 3H, CH<sub>2</sub>–CH(CH<sub>3</sub>)–CO), 1.54 (br, 2H, CH<sub>2</sub>–CH<sub>2</sub>–O), 1.44 (br, poly(*t*-butylacrylate), O–C(CH<sub>3</sub>)<sub>3</sub>), 1.25 (br, CH<sub>3</sub>–(CH<sub>2</sub>)<sub>9</sub>–CH<sub>2</sub>), 0.88 (t, J = 6.8 Hz, 3H, CH<sub>3</sub>–(CH<sub>2</sub>)<sub>9</sub>–CH<sub>2</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):

$\delta$  221.78 (C(S)-S), 174.18, 173.94 (CH(CH<sub>3</sub>)-C(O)-O), 165.78 (aryl-C(O)-O), 164.32 (C(N-R)-N), 136.80, 131.82, 131.34, 130.23, 129.94, 129.77, 126.97, 119.91, 80.34, 61.86, 61.07, 42.36, 41.97, 37.30, 36.06, 31.91, 29.62, 29.55, 29.44, 29.34, 29.10, 28.90 28.10, 28.03, 27.88, 22.69, 16.56, 14.14 (CH<sub>2</sub>-CH<sub>3</sub>);  $M_n$  = 4500 g mol<sup>-1</sup>,  $\bar{D}$  = 1.08 (SEC in THF, poly(methyl methacrylate) calibration).

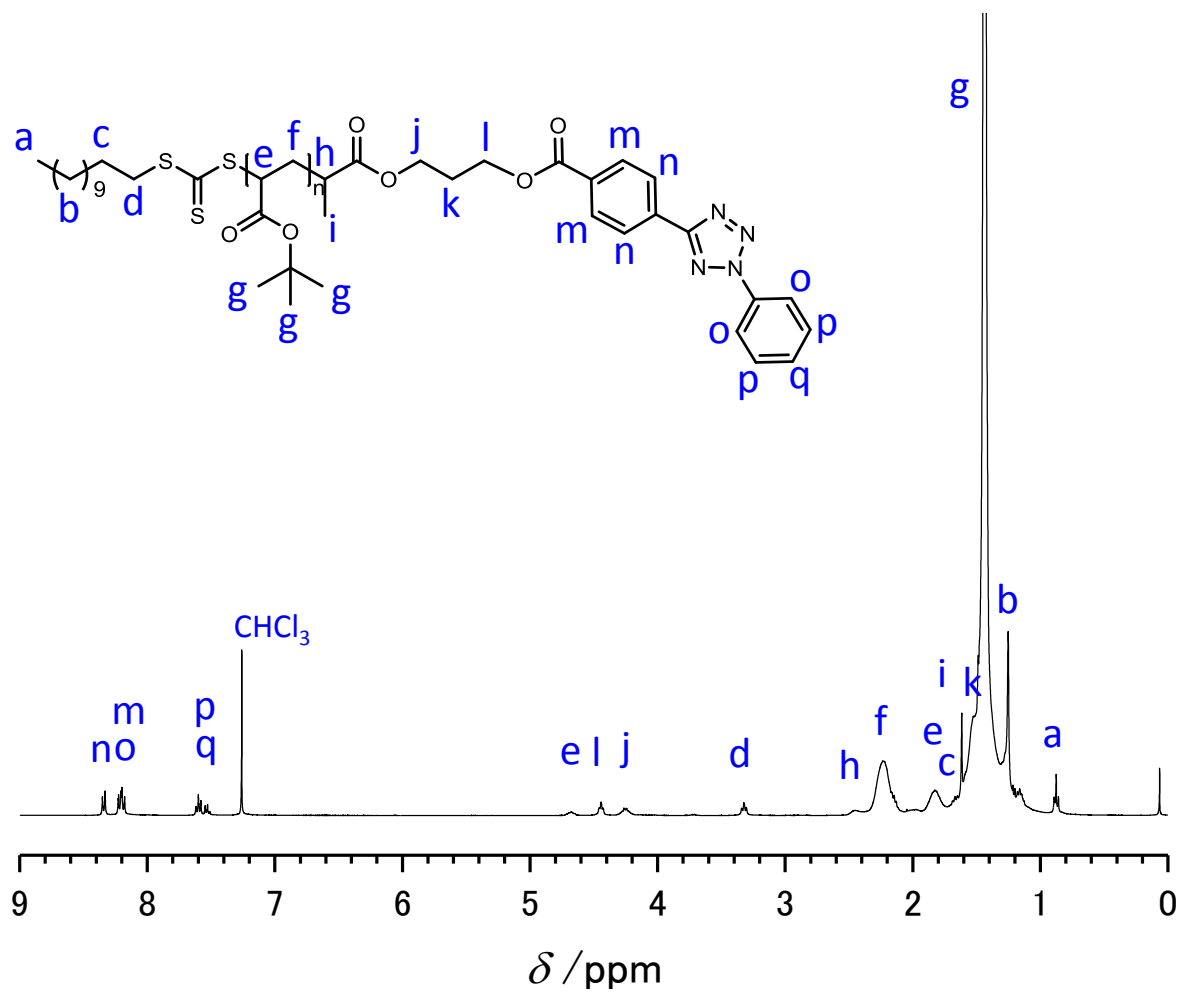


**Fig. S1** Chemical structures of 4-(2-phenyl-2H-tetrazol-5-yl)benzoic acid (**1**), tetrazole-functionalized PEG (**2**), and tetrazole-functionalized RAFT agent (**3'**).

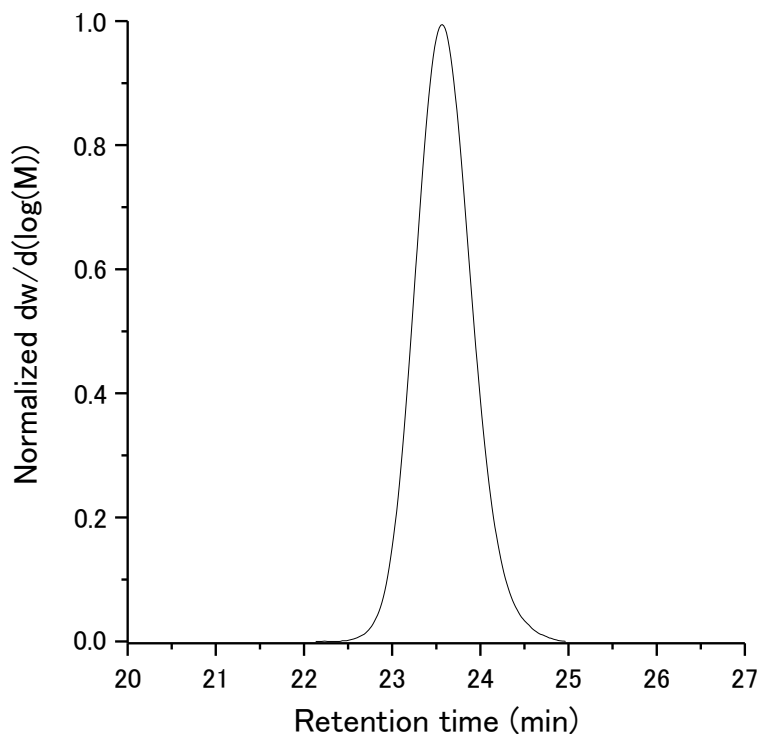


**Scheme S1** Polymerization scheme of the tetrazole-terminal poly(*tert*.-butyl acrylate) (PTBA)

(**3**)



**Fig. S2** <sup>1</sup>H NMR spectrum of **3** (400 MHz, CDCl<sub>3</sub>).

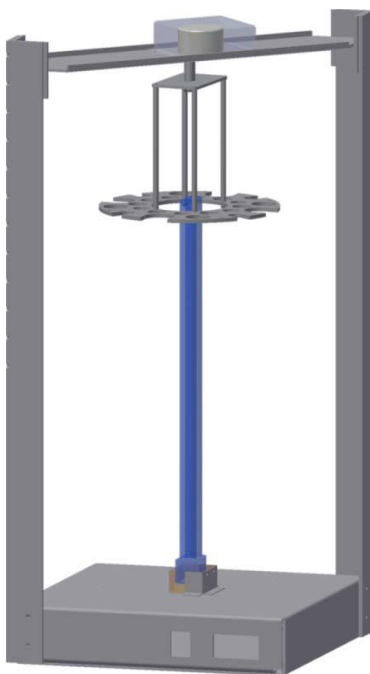


**Fig. S3** SEC trace of **3** in THF.  $M_n = 4500 \text{ g mol}^{-1}$ ,  $\bar{D} = 1.08$ .

### Irradiation equipment

The samples to be irradiated were placed into a metallic disk revolving around a compact low-pressure fluorescent lamps, Philips PL-S (Philips GmbH Market DACH, Hamburg, Germany, emitting at 290–315 nm, 9 W) for the small molecule tetrazole (**1**) and tetrazole at the terminus of the respective polymer chains (**2** and **3**). For the surface ligation, an Arimed B6 (Cosmedico GmbH, Stuttgart, Germany, emitting at 280–340 nm, 36 W) was employed. The lamp was set at a distance of 40–50 mm in a custom-built photoreactor (Fig. S4).



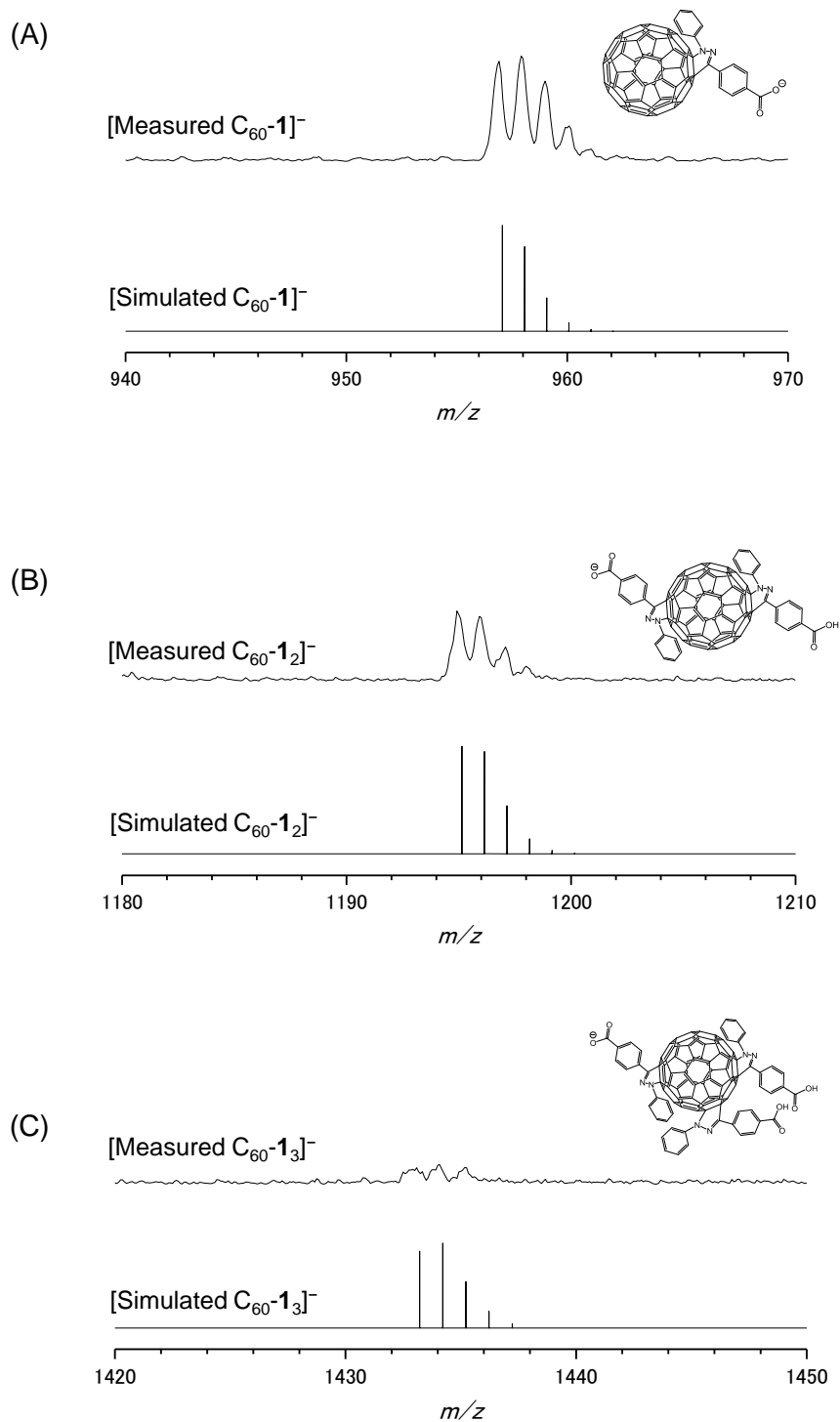


**Fig. S4** Schematic drawing of the custom-built photoreactor employed in the paper.

### **Photo-induced NITEC**

#### *Small molecule tetrazole ligation*

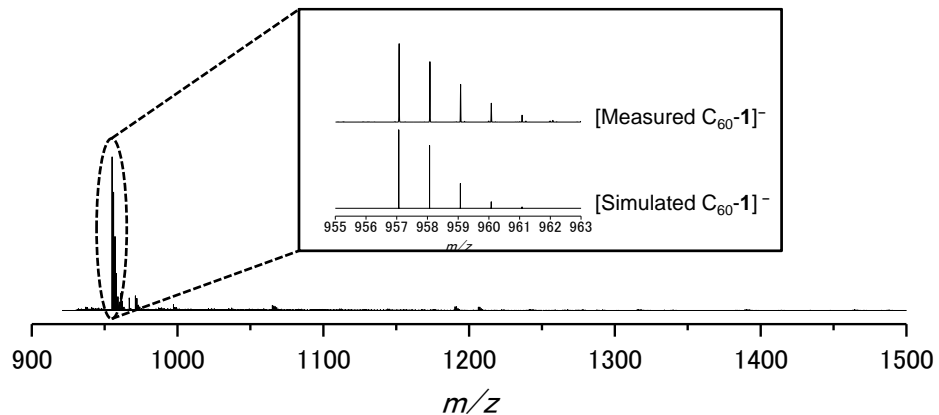
A reaction mixture containing 1 mM C<sub>60</sub> and 10 eq. (or 1 eq.) of **1** in 10 mL 1,2-dichlorobenzene was exposed to UV light (Philips PL-S, 290–350 nm) for 6 h. The ligated C<sub>60</sub>-**1** was purified by column chromatography on silica gel using toluene and toluene/ethyl acetate (50:50) with a small amount of AcOH as eluting solvent (*R<sub>f</sub>* ~ 0.4). ESI-MS measurement was performed after drying under vacuum and dissolution in THF/MeOH (3:2) with a small amount of potassium acetate. The measurements were conducted in negative mode. In the case when 1 eq. of **1** was employed, no purification was carried out before the ESI-MS (Orbitrap) measurement.



**Fig. S5** ESI-MS spectra and comparison with simulated isotopic peak patterns for (A) C<sub>60</sub>-1, (B) C<sub>60</sub>-1<sub>2</sub> and (C) C<sub>60</sub>-1<sub>3</sub>. Solvent: THF/MeOH (3:2), Salt: potassium acetate, constant spray voltage: 3.75 kV, capillary temperature: 350 °C. The measurement was conducted in negative mode.

**Table S1** Experimental and theoretical  $m/z$  values for the labeled peaks in Fig. S5

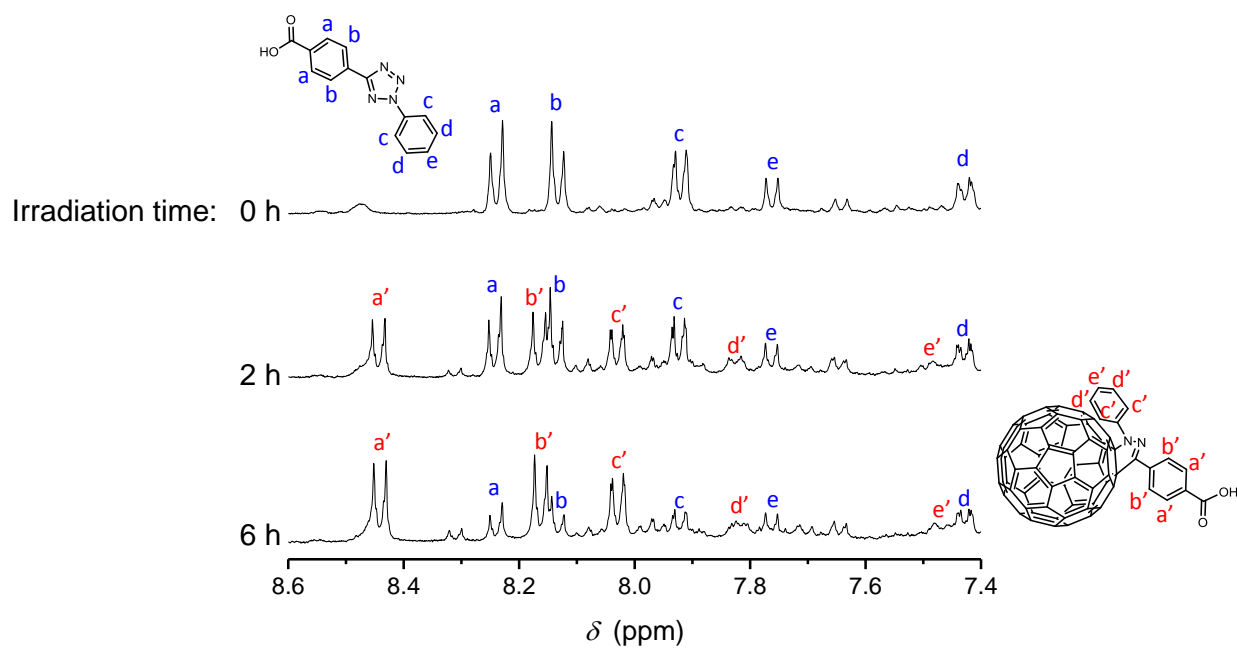
$m/z_{\text{exp}}$	assignment	formula	$m/z_{\text{theo}}$	$\Delta m/z$
956.92	C <sub>60</sub> - <b>1</b>	[C <sub>74</sub> H <sub>9</sub> N <sub>2</sub> O <sub>2</sub> ] <sup>−</sup>	957.07	0.15
1194.92	C <sub>60</sub> - <b>1</b> <sub>2</sub>	[C <sub>88</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> ] <sup>−</sup>	1195.14	0.22
1432.92	C <sub>60</sub> - <b>1</b> <sub>3</sub>	[C <sub>102</sub> H <sub>29</sub> N <sub>6</sub> O <sub>6</sub> ] <sup>−</sup>	1433.22	0.30



**Fig. S6** Orbitrap ESI-MS spectrum and comparison with simulated isotopic peak patterns representing C<sub>60</sub>-**1** without any C<sub>60</sub>-**1**<sub>2</sub> and C<sub>60</sub>-**1**<sub>3</sub>. Solvent: THF/MeOH (3:2), Salt: potassium acetate, constant spray voltage: 2.5 kV, capillary temperature: 320 °C. The measurement was conducted in negative mode. The sample was prepared using 1 eq. of **1**.

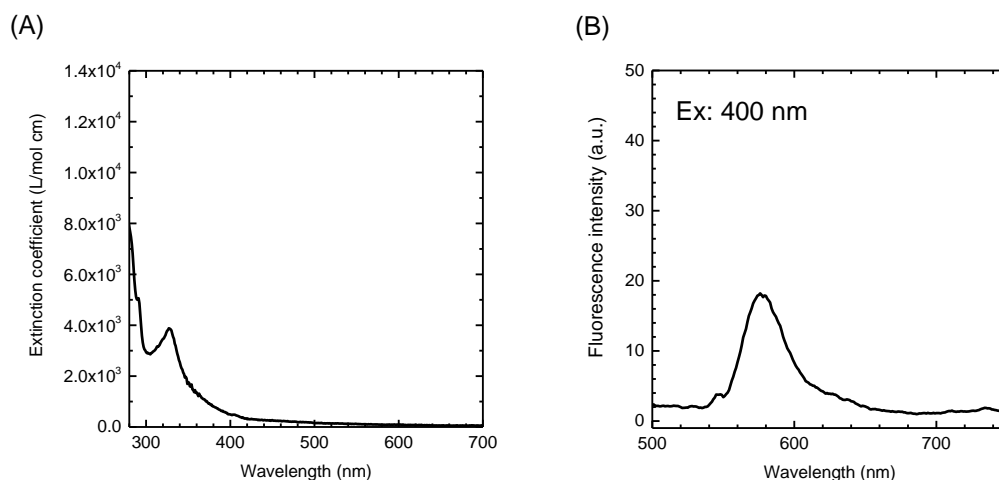


**Fig. S7** Photograph of C<sub>60</sub>-1.



**Fig. S8** <sup>1</sup>H NMR spectra of irradiated solution with C<sub>60</sub> and 1 eq. of **1** (400 MHz, toluene).

Conversion was calculated by the following equation: %conversion = integral of c'/(integral of a+integral of c') $\times$ 100 = 72%.



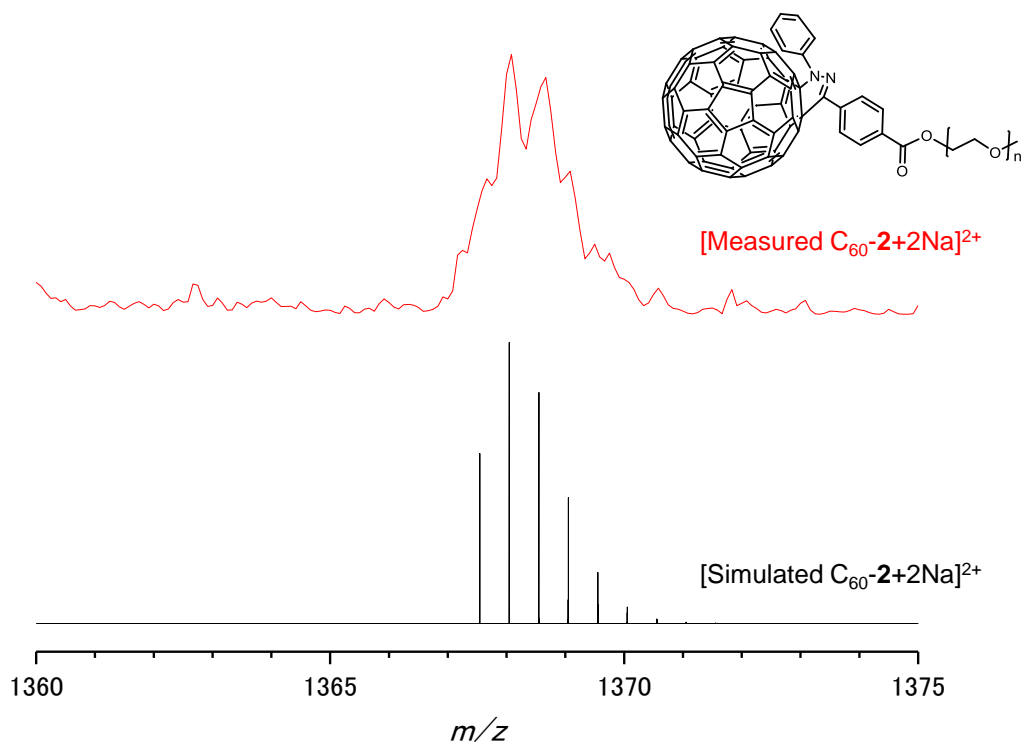
**Fig. S9** Optical analysis of C<sub>60</sub>-**1**, (A) UV-vis absorption spectrum measured in dichloromethane (concentration =  $1.4 \times 10^{-4}$  mol L<sup>-1</sup>); (B) fluorescence emission spectrum in dichloromethane. Excitation wavelength 400 nm. The spectrum was modified by smoothing using Adjacent Averaging in Origin<sup>®</sup> (Origin Lab Corp., MA, USA).

#### *Ligation to polymers carrying tetrazole chain termini*

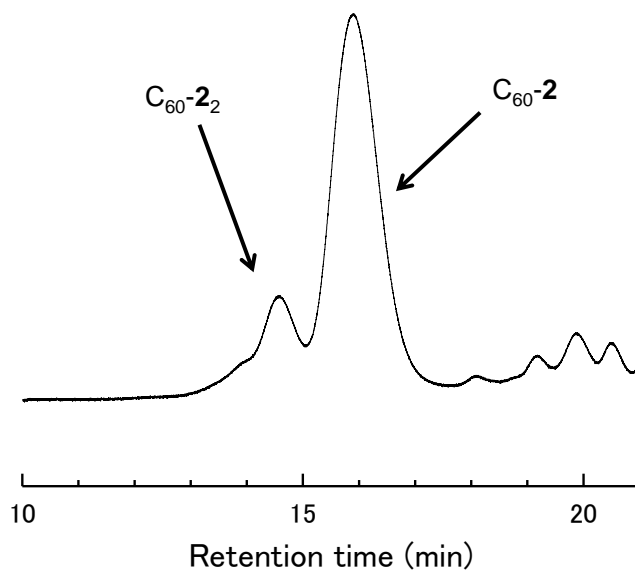
The reaction mixture containing 0.6 mM C<sub>60</sub> and 0.2 mM of **2** in 10 mL 1,2-dichlorobenzene was exposed to UV light (Philips PL-S, 290–350 nm) for 6 h. SEC/ESI-MS measurement was performed after drying under vacuum and dissolving in THF. After SEC, MeOH with sodium iodide was added before direct infusion into the ESI-MS instrument. The measurement was conducted in positive mode.

The reaction mixture containing 0.6 mM C<sub>60</sub> and 0.2 mM of **3** in 10 mL 1,2-dichlorobenzene was exposed to UV light (Philips PL-S, 290–350 nm) for 6 h. ESI-MS measurement was performed after drying under vacuum and washing with MeOH. The solvent for ESI-MS measurement is

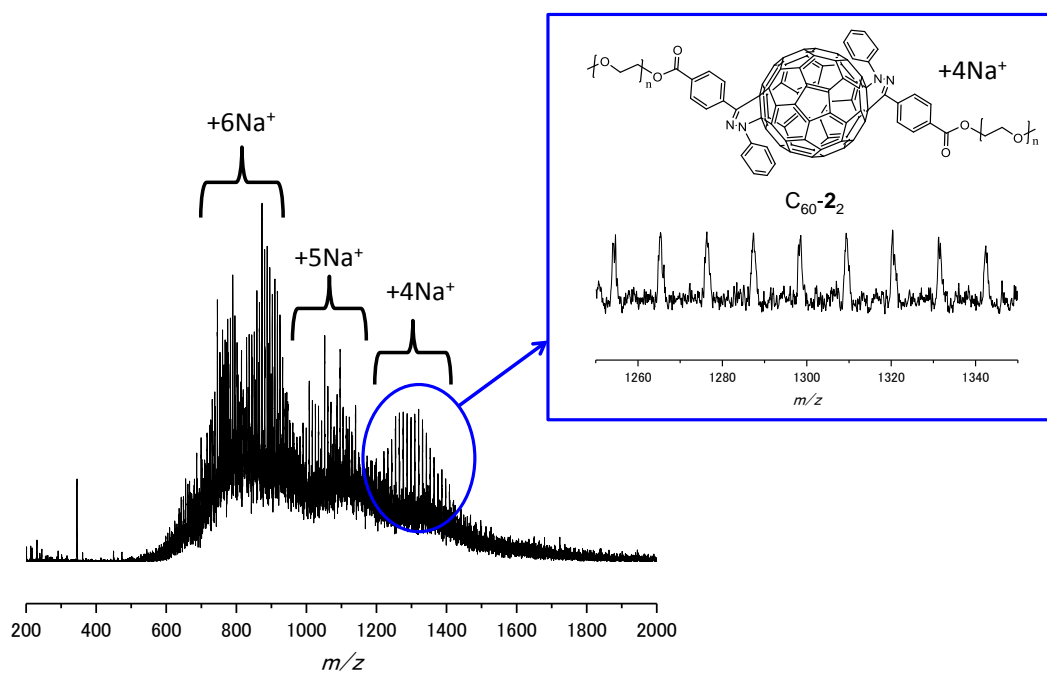
THF/MeOH (3:2) with a small amount of sodium trifluoroacetate. The measurement was conducted in positive mode.



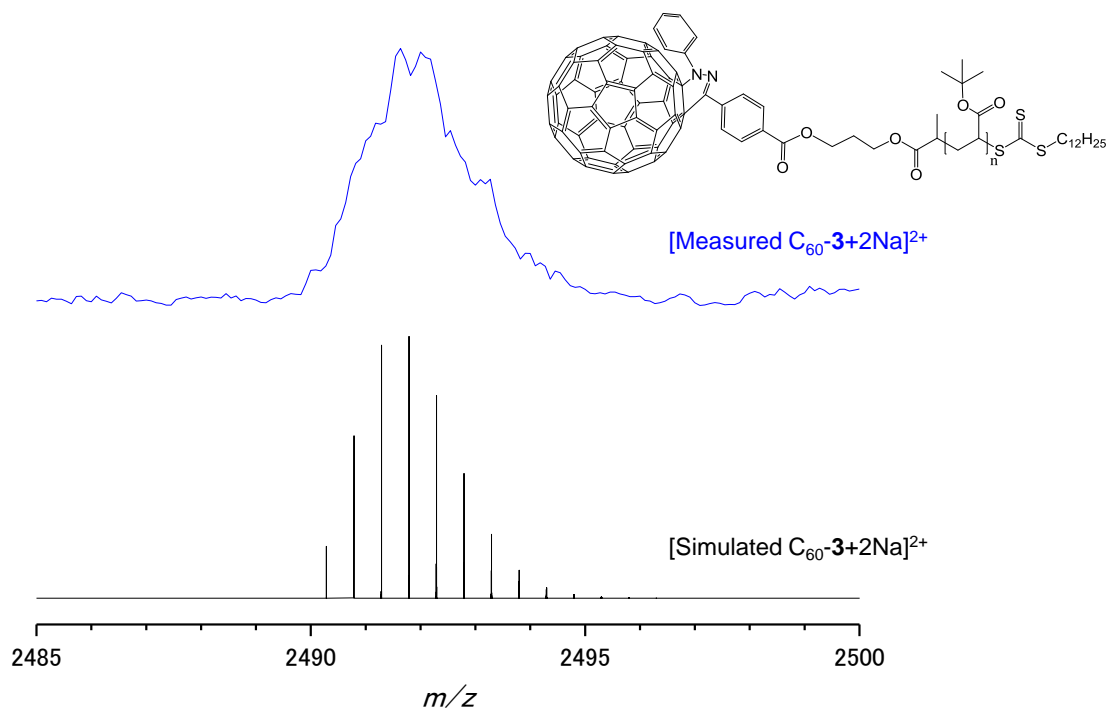
**Fig. S10** ESI-MS spectrum (top) and comparison with simulated peak pattern (bottom) for  $C_{60}-2$ . Solvent: THF/MeOH (3:2), Salt: sodium iodide, constant spray voltage: 4.5 kV, capillary temperature: 300 °C. The measurement was conducted in positive mode.



**Fig. S11** SEC diagram after NITEC ligation of fullerene with **2**.



**Fig. S12** ESI-MS spectrum and chemical structure of di-ligated fullerene,  $C_{60}-2_2$ . Solvent: THF/MeOH (3:2), Salt: sodium iodide, constant spray voltage: 4.5 kV, capillary temperature: 300 °C. The measurement was conducted in positive mode.

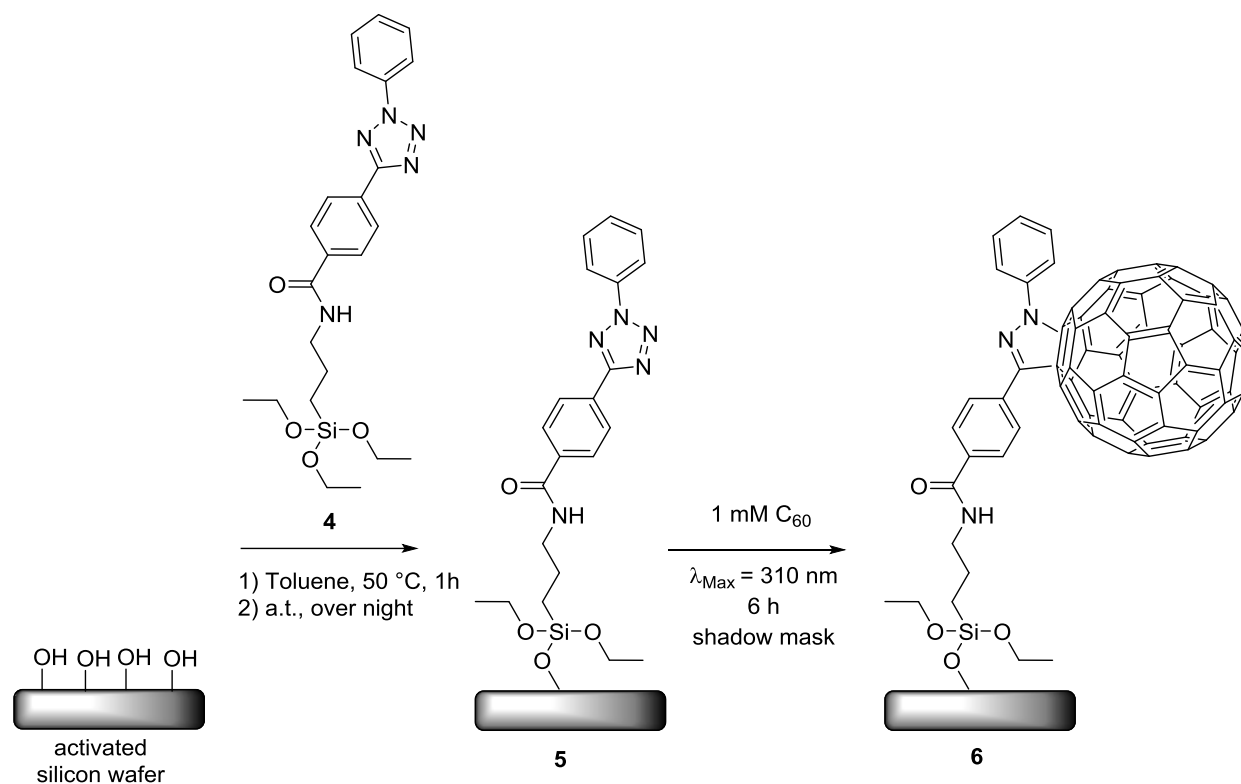


**Fig. S13** ESI-MS spectrum (top) and comparison with simulated isotopic peak pattern (bottom) for **C<sub>60</sub>-3**. Solvent: THF/MeOH (3:2), Salt: sodium trifluoroacetate, constant spray voltage: 4.5 kV, capillary temperature: 300 °C. The measurement was conducted in positive mode.

**Table S2** Experimental and theoretical  $m/z$  values for the labeled peaks in Fig. S6 and S9

$m/z_{\text{exp}}$	assignment	formula	$m/z_{\text{theo}}$	$\Delta m/z$
1368.08	<b>C<sub>60</sub>-2</b> (n = 39)	$[\text{C}_{153}\text{H}_{168}\text{N}_2\text{Na}_2\text{O}_{41}]^-$	1368.05	0.03
2491.73	<b>C<sub>60</sub>-3</b> (n = 28)	$[\text{C}_{289}\text{H}_{380}\text{N}_2\text{Na}_2\text{O}_{60}\text{S}_3]^-$	2491.79	0.06





**Scheme S2** Synthetic route for spatially resolved immobilization of C<sub>60</sub> on silicon.

*Preparation of the tetrazole functionalized silicon wafer (5)*

The surface (**5**) and the employed tetrazole silane agent (**4**) were prepared as previously reported.<sup>5</sup> The neat silicon wafer was activated in a 1:2 mixture of H<sub>2</sub>O<sub>2</sub> and conc. H<sub>2</sub>SO<sub>4</sub> for 2 h at 100 °C. The wafer was extensively washed with MilliQ water and dried in an argon stream. Subsequently, the activated wafer was covered with a solution of silane **4** in dry toluene (5 mg/mL), heated to 50°C for 1 h and stored at ambient temperature overnight. Unreacted silane was removed by washing with toluene (HPLC grade), and the wafer (**5**) dried in an argon stream.

### *Spatially resolved immobilization of C<sub>60</sub> onto tetrazole functionalized silicon wafer (6)*

The tetrazole functionalized silicon wafer (**5**) was covered with a dot shadow mask, placed into a sample holder and immersed in a 1 mM fullerene solution in dry 1,2-dichlorobenzene. The system was exposed to UV light (Arimed B6 lamp,  $\lambda_{\text{max}} = 310$  nm) for 5 min or 6 h and subsequently washed with large amounts toluene (HPLC grade). The ToF-SIMS image of **6**, which verifies the covalent and spatially resolved attachment of C<sub>60</sub>, is depicted in Fig. 4B and C.

### References

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