

1 **Types of paper**

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4 **Title**

5 Preparation of 6-azafulleroid-6-deoxy-2,3-di-*O*-myristoylcellulose

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20 **Abstract**

21 6-Azafulleroid-6-deoxy-2,3-di-*O*-myristoylcellulose (**3**) was synthesized from 6-azido-6-deoxycellulose (**1**) by
22 two reaction steps. The myristoylation of compound **1** with myristoyl chloride / pyridine proceeded smoothly to
23 give 6-azido-6-deoxy-2,3-di-*O*-myristoylcellulose (**2**) in 97.0 % yield. The reaction of compound **2** with fullerene
24 (C₆₀) was carried out by microwave heating to afford compound **3** in high yield. It was found from FT-IR, ¹³C-
25 NMR, UV-vis, differential pulse voltammometry (DPV), SEC analyses that compound **3** was the expected C₆₀-
26 containing polymer. Consequently, maximum degree of substitution of C₆₀ (DS_{C60}) of compound **3** was 0.33.

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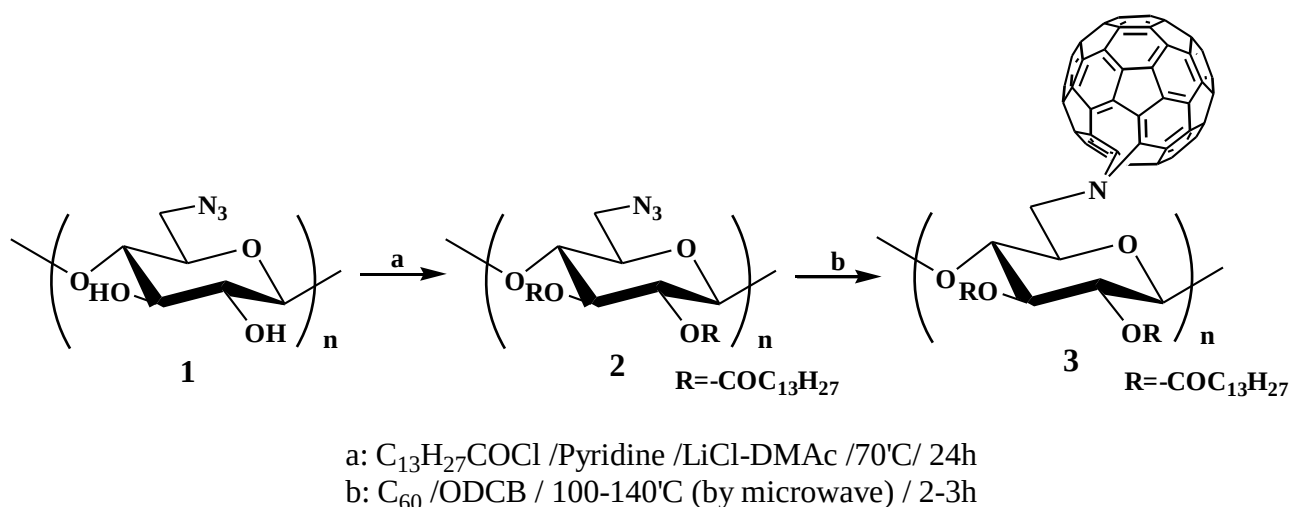
28 **Keywords**

29 Azafulleroid, Cellulose, Fullerene, Microwave heating

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Cellulose is the most abundant biomacromolecule in nature, and is important as biodegradable and renewable organic material. Recently, new applications of cellulose derivatives as advanced materials such as shape memory-recovery material,¹ and photoactive materials,² have been reported. One of the proposals of cellulose derivatives for the advanced materials is the photocurrent generation system using porphyrin-containing cellulose derivatives as electron donor materials.³⁻⁵ Sakakibara and Nakatsubo reported the Langmuir-Blodgett film of porphyrin-fullerene (C₆₀) system using the porphyrin-containing cellulose derivative and C₆₀ with high photocurrent generation performance.⁴ Then, C₆₀-containing cellulose derivative is also attractive for the photocurrent generation system as an electron acceptor material, because it is expected to be useful for forming an electron transporting pathway in the system. However, there is no report for the preparation of C₆₀-containing cellulose derivative. Addition reaction of organic azides with C₆₀ has been widely applied to the preparation of C₆₀-bearing polymers.⁶⁻¹² Then, this paper describes the preparation of 6-azafulleroid-6-deoxy-2,3-di-O-myristoylcellulose (**3**) from 6-azido-6-deoxycellulose (**1**). In the target compound **3**, myristoyl group was selected as O-2 and O-3 substituent groups to enhance solubility for common organic solvents and formability of Langmuir-Blodgett film, because it was found to be preferable to the purposes in a preliminary experiment.

The synthetic route for 6-azafulleroid-6-deoxy-2,3-di-O-myristoylcellulose (**3**) from 6-azido-6-deoxycellulose (**1**)¹³ by two reaction steps is shown in Scheme 1. Myristoylation of 6-azido-6-deoxycellulose (**1**) with myristoyl chloride in the presence of pyridine in LiCl /DMAc afforded 6-azido-6-deoxy-2,3-di-O-myristoylcellulose (**2**) in 97.0 % yield.



Scheme 1 Synthetic route for 6-azafulleroid-6-deoxy-2,3-di-O-myristoyl cellulose (**3**)

Addition reaction of C₆₀ to compound **2** was carried out according to the modified method of Okamura et al. to give 6-azafulleroid-6-deoxy-2,3-di-O-myristoyl cellulose (**3**).⁹ That is, compound **2** and C₆₀ were reacted at

140 °C for 3 h in *o*-dichlorobenzene (ODCB) to give product **3-i**. Microwave (MW) heating was used for the reaction because it was reported that MW heating has an advantage of shortening reaction times compared with conventional heating (an oil bath method) in the preparation of cellulose derivatives¹⁴ and in the addition reaction of C₆₀ to azido-compounds.^{12,15,16}

Product **3-i**, which was easily soluble in organic solvents such as CHCl₃, CH₂Cl₂, THF, toluene, chlorobenzene and ODCB, was subjected to FT-IR, ¹³C-NMR, UV-vis, differential pulse voltammometry (DPV) and SEC measurement for its characterization. In FT-IR spectrum of product **3-i**, the band at 2104 cm⁻¹ from azido groups was completely disappeared, suggesting that heating time for 3 h by microwave heating was enough for the addition reaction. The small characteristic band at 527 cm⁻¹ derived from C₆₀^{8,10} was newly appeared. In ¹³C-NMR spectrum of product **3-i**, the broad peak in the range of 130 to 150 ppm assigned to C₆₀ moiety^{9,11} and the sharp peaks in the range of from 17 to 35 ppm derived from myristoyl groups were observed. Fig.1 shows UV-vis spectrum of product **3-i** and C₆₀. The characteristic peaks at 330 nm from C₆₀^{7,10} were found in the spectrum of product **3-i**, although compound **2** has no absorption at the region. Electrochemical analysis such as cyclic voltammetry (CV) and differential pulse voltammometry (DPV) is one of the methods for characterization of substituted C₆₀. It is reported that the reduction potential peaks, which are observed in CV or DPV of unsubstituted C₆₀, are negatively shifted in CV or DPV of substituted C₆₀ such as azafulleroid.^{10,17,18} Figure 2 shows the DPV curves of product **3-i** and C₆₀ in 0.1 M tetrabutylammonium perchlorate (TBAP) / ODCB. The negative shifts of three characteristic reduction peaks of C₆₀ were observed in DPV of product **3-i**. SEC is also important method for characterization of C₆₀-containing polymer. For example, Okamura et al. reported that C₆₀-pullulan derivatives were characterized by SEC with RI and UV (detective wavelength: 700 nm) detections.⁹ Figure 3 shows SEC elution curves of product **3-i** by RI and UV detectors. UV detection was performed by UV-600 nm, because of the detection ability of our UV-detector. The RI and UV elution curves showed nearly identical elution profiles. All data suggested that product **3-i** was the desired C₆₀-containing cellulose derivative.

Figure 4 shows thermal gravimetric analysis (TGA) curve of product **3-i**. The thermolysis of product **3-i** started at 205°C, suggesting that product **3-i** had a aza-bridged structure, but not triazol-bridged structure, because Ungurenasu and Pinteala reported that the thermolyses of aza-bridged type C₆₀-curdian derivatives started at 205°C.¹¹ There are two possibilities concerning aza-bridged types between nitrogen at C-6 position of the cellulose derivative and C₆₀, that is, [6,6]-close type and [5,6]-open type,^{15,16,18-22} although it is reported that alkyl azides predominantly added at the [5,6]-open junction.^{19,22} The absence of the peak at 425 nm, which is a characteristic peak of [6,6]-close aza substructure,^{15,16,20} indirectly suggested that product **3-i** had a [5,6]-open

type structure. This is also supported by ^{13}C -NMR data. It is reported that the absence of the peak around 84 ppm accounted for a [5,6]-open type structure in ^{13}C -NMR spectrum of C_{60} -curdlan derivative.¹¹ Indeed, no peaks were observed in the range of 80 to 90 ppm in ^{13}C -NMR spectrum of product **3-i**.

The TGA method is widely used for determination of the weight percent of C_{60} in C_{60} -bearing polymer.^{6,7}
¹⁰ The degree of substitution of C_{60} ($\text{DS}_{\text{C}_{60}}$) of product **3-i** was calculated from TGA method, that is, it was determined using the weight change values of compounds **2** and **3-i** at 600 °C, and was found to be 0.25. The low $\text{DS}_{\text{C}_{60}}$ suggested that multi-addition of azido groups of compound **2** with C_{60} might proceed, although further investigation is required. The degree of polymerization (DP_n) of product **3-i** was determined from SEC, and was found to be 14.5. The DP_n of product **3-i** was significantly lower than that of compound **2** ($\text{DP}_n = 78.3$), suggesting that depolymerization occurred under the reaction conditions for product **3-i**.

Then, addition reaction of C_{60} with compound **2** was carried out under various conditions with different concentration, amount of C_{60} , reaction time, temperature and so on to investigate the influence of the reaction conditions to $\text{DS}_{\text{C}_{60}}$ and DP_n of the products and to get compound **3** with higher $\text{DS}_{\text{C}_{60}}$. The results are shown in Table 1. The reaction conditions for product **3-i** (Entry 1) are regarded as criteria for the various reaction conditions.

The $\text{DS}_{\text{C}_{60}}$ of the products increased with increasing of the concentration of compound **2** (Entries 1-4) and with increasing of the amount of C_{60} (Entries 1, 5-9), but leveled off when the concentration was 25 mM and when the amount of C_{60} was 2 eq, respectively. The DP_n of the products was not affected by the concentration of compound **2**, but it slightly decreased with increasing of the amount of C_{60} . The $\text{DS}_{\text{C}_{60}}$ of the products did not increase but the DP_n decreased with an increase of reaction time (Entries 1, 10-11). It was found that the band at 2104 cm^{-1} from azido groups was completely disappeared after 1.5 h by the monitoring experiment of the reaction (Entry 1) (data not shown). The $\text{DS}_{\text{C}_{60}}$ of the products increased and leveled off, but the DP_n decreased with an increase of reaction temperature (Entries 1, 12-13). Control experiments without addition of C_{60} , that is, microwave heating treatment of compound **2** with different temperature, were performed (Entries C1-C4). DP_n of the products clearly decreased with an increase of reaction temperature, especially at 180°C, which is corresponded to the boiling point of the solvent (ODCB), serious degradation of compound **2** was confirmed by FT-IR analysis. It was found that high reaction temperature was responsible for decreasing of DP_n of the products, although it was favorable to high $\text{DS}_{\text{C}_{60}}$. Product **3-xii**, prepared at 100 °C for 3 h, was insoluble in the solvents for product **3-i** such as CHCl_3 , CH_2Cl_2 , THF, toluene, ODCB, product **3-xiii**, prepared at 130 °C for 3 h, became to be partially insoluble in the solvents two weeks later, while product **3-i**, prepared at 140 °C for 3 h, was easily soluble

83 in the solvents two months later. These results suggest that higher DPn of the products **3** were undesirable to the
84 solubility of the products **3**. The DS_{C60} of the product **3-xiv**, which was prepared at 140°C for 48 h by oil-bath
85 heating, was higher than that of product **3-i**, but the DPn of product **3-xiv** was almost same as that of product **3-i**
86 (Entries 1, 14). MW heating had an advantage of only a shortening of reaction time as expected. Considering the
87 results described above, the addition reaction was carried out under the optimal reaction conditions for higher
88 DS_{C60} to afford product **3-xv** with maximum DS_{C60} of 0.33 and with DPn of 17.9 in 68.5% yield (Entry 15). It was
89 thought that C₆₀ was too bulky to be introduced to the cellulose derivative with DS_{C60} of more than 0.33 by its
90 steric hindrance.

91

92 **1. Experimental**

93 **1.1. General**

94 6-Azido-6-deoxycellulose (**1**) with DS_{N3} 0.88 was prepared according to the method of Matsui et al.¹³ Fullerene-
95 C₆₀ (98%) was purchased from Sigma-Aldrich (Tokyo, Japan) and all other chemicals were purchased from
96 commercial sources and used without further purification.

97 FT-IR spectra were recorded in KBr pellets with a Shimadzu FTIR-8600 spectrophotometer. ¹H- and ¹³C
98 NMR spectra were recorded with a Varian INOVA300 FT-NMR (300 MHz) spectrometer with TMS as an internal
99 standard in CDCl₃. Chemical shifts (δ) are given in δ values (parts per million). The UV-vis spectra were recorded
100 on a Jasco V-560 UV-vis spectrophotometer in CH₂Cl₂. Differential pulse voltammetry (DPV) measurements were
101 performed in a MCA micro cell (BSA, Japan) at room temperature at scan rate of 100 mVs⁻¹ using a platinum
102 electrode (1.6 mm diameter) as working electrode, Ag / AgCl (saturated KCl) as reference electrode, platinum
103 wire as counter electrode by an ALS electrochemical analyzer (ALS650B). Ferrocene (Fc) was added as an
104 internal standard. All potentials are given relative values to the ferrocenium / ferrocene couple (Fc⁺ / Fc). The
105 electrolyte (0.1 M TBAP in ODCB) was degassed with nitrogen before use. SEC analyses were performed using a
106 Shimadzu LC-10 system equipped with a Shimadzu UV-vis detector (SPD-10AVp) and a Shimadzu RI detector
107 (RID-10A) (Conditions: column: KF-802.5 + KF-805, column temperature: 40 °C, eluent: THF, flow rate: 1.0
108 ml/min; standards; polystyrene standards (Shodex)). TGA was conducted in nitrogen with a Shimadzu TGA-50
109 thermal analyzer by heating from 100 to 700 °C at the programming rate of 10 °C min⁻¹.

110

111 **1.2. 6-Azido-6-deoxy-2,3-di-O-myristoylcellulose (2)**

112 LiCl (1.2 g, 28.3mmol) was added to a suspension of 6-azido-6-deoxycellulose (**1**) (150 mg, 0.78mmol) in *N,N*-

113 dimethylacetamide (15 ml) at 60 °C. The reaction mixture became a clear solution within was several minutes.
 114 Pyridine (1.3 ml, 16.2mmol) and myristoyl chloride (2.18 ml, 8.04 mmol) were added to the solution. After
 115 stirring at 70 °C for 24 h, the solution was diluted with CH₂Cl₂. The organic layer was washed with 1 M HCl,
 116 water and brime, dried over Na₂SO₄ and concentrated in vacuo to give an oil. The solution of the oil in a small
 117 amount of CH₂Cl₂ was dropped into EtOH (500 ml). The resulting precipitate was collected by centrifugation
 118 (15000 rpm, 15 minutes), and was purified by the re-precipitation method again to give 6-azido-6-deoxy-2,3-di-O-
 119 myristoylcellulose (**2**) as a brown solid (470 mg, 97.0% yield).
 120 Compound **2**; DS_{myristoyl}: 2.02 (determined by elementary analysis); DPn: 78.3 (*Mw/Mn*: 3.36); ¹H NMR (CDCl₃):
 121 δ 5.13 (H-3), 4.76 (H-2), 4.50 (H-1), 3.75 (H-4), 3.61 (H-5,6a), 3.41 (H-6b), 2.23
 122 (OC(=O)CH₂CH₂C₁₀H₂₀CH₃), 1.53 (OC(=O)CH₂CH₂C₁₀H₂₀CH₃), 1.26, (OC(=O)CH₂CH₂C₁₀H₂₀CH₃),
 123 0.88 (OC(=O)CH₂CH₂C₁₀H₂₀CH₃) ppm; ¹³C NMR (CDCl₃): δ 172.5, 171.8 (C=O), 99.7 (C-1), 75.0–71.8 (C-
 124 2,3,4,5), 50.0 (C-6), 33.9, 31.9, 29.6, 24.7, 22.7 (OC(=O)C₁₂H₂₄CH₃), 14.1 (OC(=O)C₁₂H₂₄CH₃) ppm; FT-IR
 125 (KBr): ν 2104 ((N₃), 1757 (C=O) cm⁻¹.
 126

127 1.3. 6-Azafulleroid-6-deoxy-2,3-di-O-myristoylcellulose (**3**)

128 *Typical method* - 6-Azido-6-deoxy-2,3-di-O-myristoylcellulose (**2**) (30 mg, 0.050 mmol) was reacted with
 129 fullerene (32 mg, 0.044 mmol) in ODCB (5 ml) at 140 °C for 3 h in a 10 ml-test tube by microwave heating with
 130 a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC), which consists of a continuous focused microwave
 131 power delivery system with power output from 0 to 300 W at 2.45 GHz. The reaction mixture was purified by a
 132 silica gel column eluted firstly with toluene to remove unreacted C₆₀ and secondly with THF to be recovered, and
 133 concentrated in vacuo to give a crude product. The solution of the product in a small amount of CH₂Cl₂ was
 134 dropped into MeOH (200 ml). The resulting precipitate was collected by centrifugation (15000 rpm, 15 minutes),
 135 and was purified by the re-precipitation method again to give 6-Azafulleroid-6-deoxy-2,3-di-O-myristoylcellulose
 136 (**3-i**) (31.9 mg, 85.1 %yield).

137 Compound **3-i**; DS_{C₆₀}: 0.25 (determined by TGA method); DPn: 14.5 (*Mw/Mn*: 3.64); ¹³C NMR (CDCl₃): δ 172.4
 138 (C=O), 150 – 130 (C₆₀), 68–76 (C-2,3,4,5), 34.2, 32.2, 30.0, 25.0, 23.0, (OC(=O)C₁₂H₂₄CH₃), 14.4
 139 (OC(=O)C₁₂H₂₄CH₃) ppm; FT-IR (KBr) : ν 1755 (C=O), 527 (C₆₀) cm⁻¹.
 140
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Captions (Scheme, Figures and Table)

Scheme 1 Synthetic route for 6-azafulleroid-6-deoxy-2,3-di-*O*-myristoylcellulose (**3**)

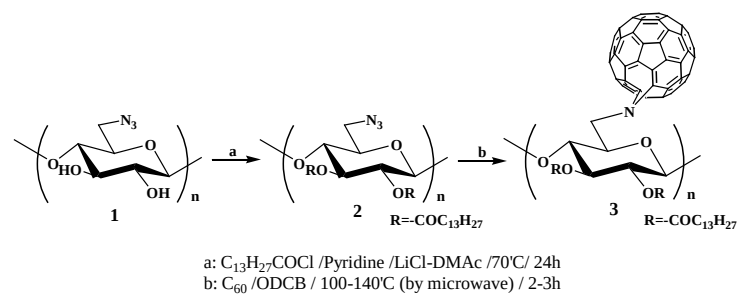
Figure 1 UV-vis spectra of product **3-i** and C₆₀

Figure 2 DPVs of product **3-I**, C₆₀ and compound **2**

Figure 3 SEC elution curves of product **3-i**

Figure 4 TGA curves of product **3-i** and C₆₀

Table 1 Results of addition reaction of C₆₀ to compound **2** under various reaction conditions



Scheme 1 Synthetic route for 6-azafulleroid-6-deoxy-
 2,3-di-O-myristoylcellulose (**3**)

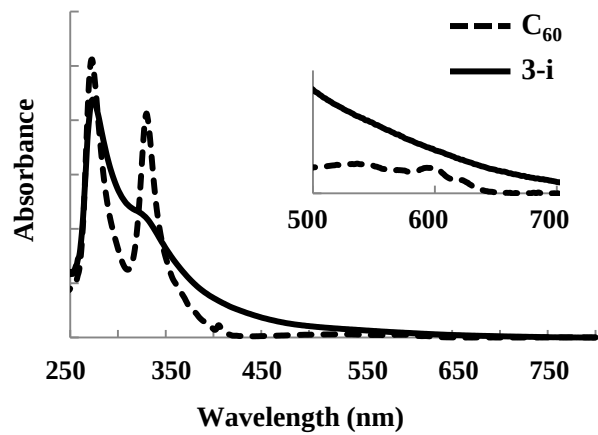


Figure 1 UV-vis spectra of product **3-i** and C_{60}

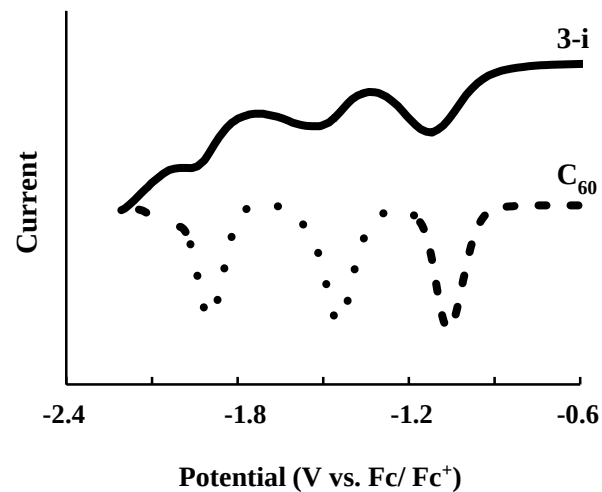


Figure 2 DPVs of product **3-i** and C_{60}

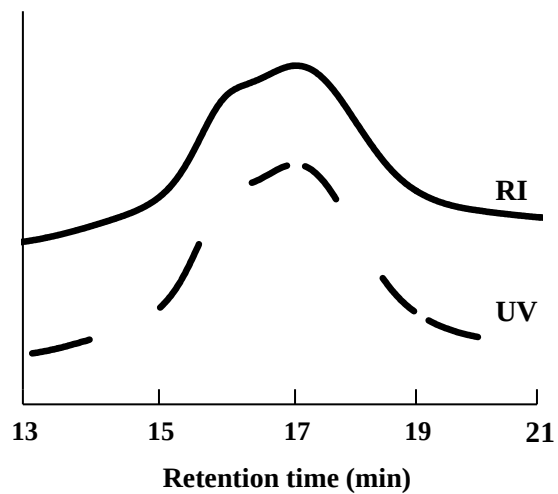


Figure 3 SEC elution curves of product **3-i**

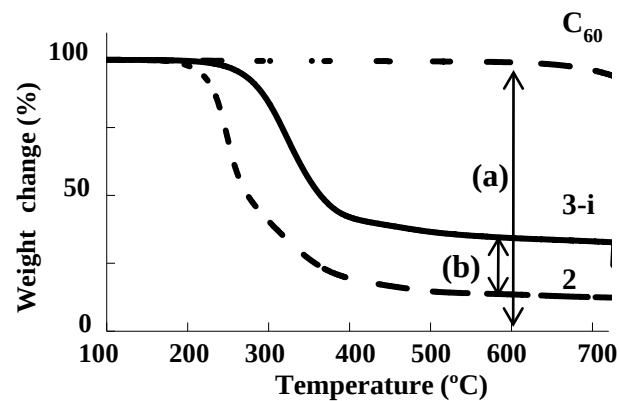


Figure 4 TGA curves of product **3-i**, C_{60} and compound **2**

Table 1 Results of addition reaction of C₆₀ to compound 2 under various reaction conditions

Entry	Concentration of 2 (mM)	Amount of C ₆₀ ^{a)} (eq)	Time (h)	Temperature (°C)	Heating method ^{b)}	Product	DS _{C60} ^{c)}	DPn	Mw/Mn
1	10	1	3	140	MW	3-i	0.25	14.5	3.64
2	5	1	3	140	MW	3-ii	0.21	13.0	2.66
3	20	1	3	140	MW	3-iii	0.28	13.2	3.04
4	25	1	3	140	MW	3-iv	0.30	15.9	4.15
5	10	0.1	3	140	MW	3-v	0.11	18.3	4.52
6	10	0.2	3	140	MW	3-vi	0.16	15.8	3.84
7	10	2	3	140	MW	3-vii	0.30	13.5	4.28
8	10	4	3	140	MW	3-viii	0.28	15.3	6.57
9	10	6	3	140	MW	3-ix	0.31	14.4	4.49
10	10	1	1	140	MW	3-x	0.23	19.7	6.26
11	10	1	2	140	MW	3-xi	0.27	18.1	4.72
12	10	1	3	100	MW	3-xii	0.14	n.m. ^{d)}	n.m. ^{d)}
13	10	1	3	130	MW	3-xiii	0.28	21.6	17.2
14	10	1	48	140	oil bath	3-xiv	0.32	13.2	3.21
15	25	2	2	140	MW	3-xv	0.33	17.9	3.46
C1	10	0	3	100	MW	2-i	-	70.9	2.58
C2	10	0	3	120	MW	2-ii	-	49.6	2.64
C3	10	0	3	140	MW	2-iii	-	16.5	2.57
C4	10	0	3	180	MW	2-iv	-	n.m. ^{d)}	n.m. ^{d)}

a) per N₃-group b) MW = microwave c) DS_{C60} were calculated by TGA method.

d) n.m. = Not measured (because the product was insoluble)