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Article

Synthesis and Characterization of Positively Charged Pentacationic [60]Fullerene Monoadducts for Antimicrobial Photodynamic Inactivation

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Abstract: We designed and synthesized two analogous pentacationic [60]fullerenyl monoadducts, C₆₀(>ME₁N₆⁺C₃) (**1**) and C₆₀(>ME₃N₆⁺C₃) (**2**), with variation of the methoxyethyleneglycol length. Each of these derivatives bears a well-defined number of cationic charges aimed to enhance and control their ability to target pathogenic Gram-positive and Gram-negative bacterial cells for allowing photodynamic inactivation. The synthesis was achieved by the use of a common synthon of pentacationic *N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amine arm (C₃N₆⁺) having six attached propyl groups, instead of methyl or ethyl groups, to provide a well-balanced hydrophobicity–hydrophilicity character to pentacationic precursor intermediates and better compatibility with the highly hydrophobic C₆₀ cage moiety. We demonstrated two plausible synthetic routes for the preparation of **1** and **2** with the product characterization via various spectroscopic methods.

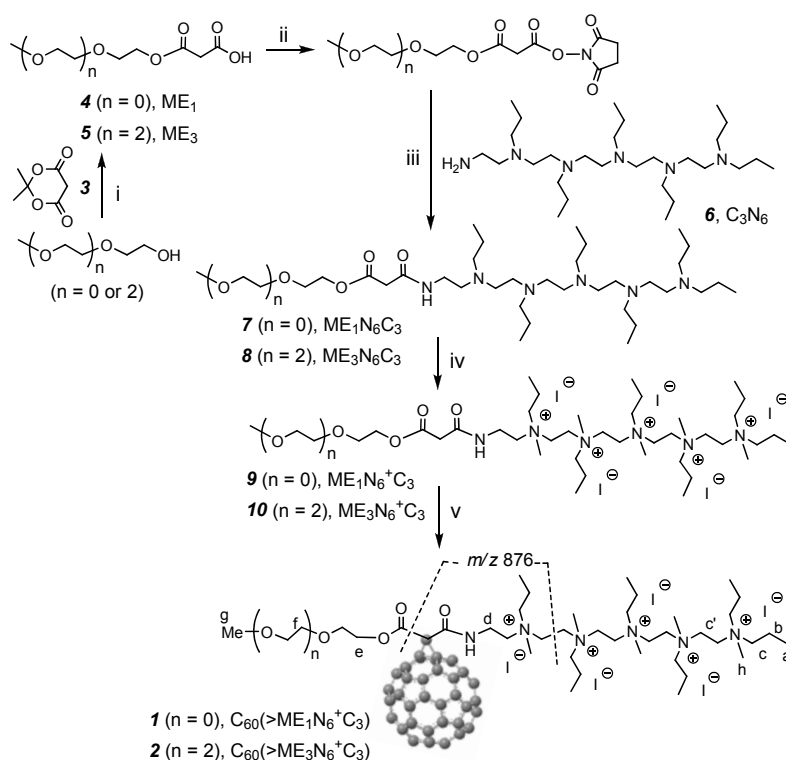
Keywords: pentacationic C₆₀ monoadducts; decacationic C₆₀ monoadduct; *N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amine; photosensitizer

1. Introduction

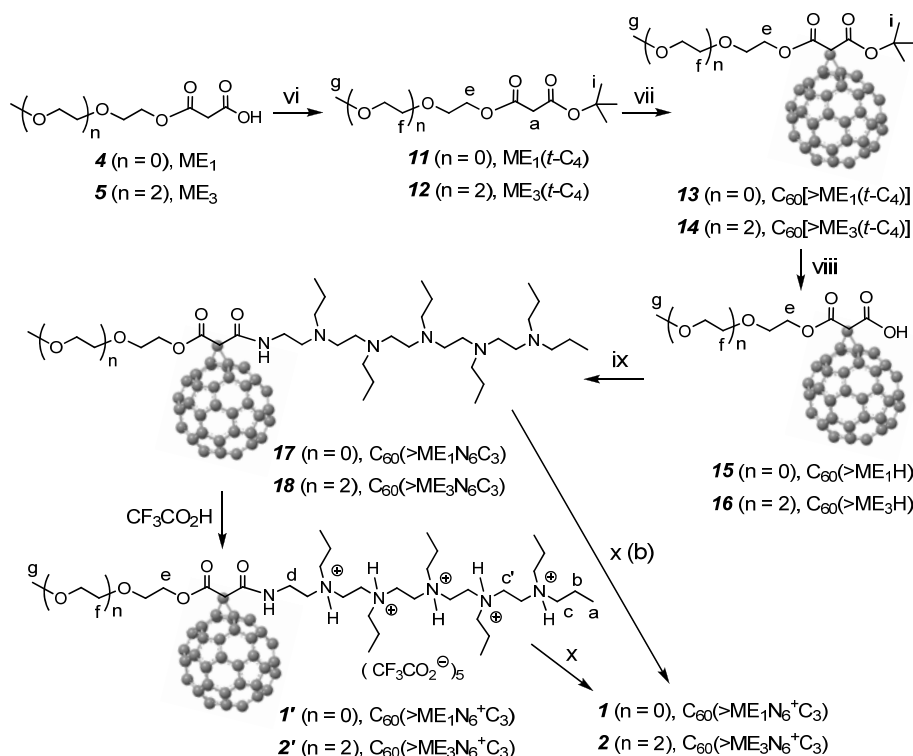
Broad-spectrum one-photon based photodynamic therapy (1 γ -PDT)-mediated killing of pathogenic Gram-positive (e.g., *Staphylococcus aureus*) and Gram-negative (e.g., *Escherichia coli*) bacterial targets using a conventionally accessible light source is an emerging medical approach to treat infectious diseases, especially, those caused by multi-antibiotic-resistant bacteria [1–5]. The efficacy of 1 γ -PDT depends on several parameters, including photophysical characteristics of the photosensitizer, the ability of the photosensitizer to target bacteria, the method of administration, and the availability of an appropriate light source. Fullerenes are highly photostable molecules suitable for single-dose multiple-treatments applications. Nearly quantitative efficiency of intersystem crossing from the excited singlet state of [60]fullerene ($^1C_{60}^*$) to its triplet excited state ($^3C_{60}^*$) readily allows intermolecular triplet energy transfer from $^3C_{60}^*$ to molecular oxygen leading to the production of singlet oxygen (1O_2) [6,7], which is highly reactive toward biological substrates producing subsequent cell damage. This photochemical mechanism serves as the basis of photodynamic cytotoxicity against pathogenic microorganisms, including multi-antibiotic-resistant bacteria. However, chemical functionalization of C_{60} is necessary to enhance its solubility in water. In general, attachment of multiple hydroxyl, carboxylic acid, and glycolic oxide addend groups may serve the purpose. Water-solubility of these derivatives increases as the number of hydrophilic groups increases, whether these functional groups are located in either the same addend group or different addend moieties. The latter case leads to the synthesis of fullereryl multiadducts that may change significantly the molecular orbital configuration of the fullerene cage and, thus, its HOMO–LUMO energy gap level and effectiveness in the production of 1O_2 . The 1 γ -PDT efficiency can be optimized by performing only a limited number of addition reactions to the fullereryl olefinic bonds to preserve the low HOMO–LUMO energy gap level of the cage. This restriction suggests that [60]fullerene monoadducts using hydrophilic or amphiphilic groups would be suitable candidates. However, since only one addend group is able to be attached to the cage in a monoadducts, sufficient hydrophilicity is required to allow compatibility of the resulting derivative with water.

It has been demonstrated that polycationic photosensitizers exhibited high activity as 1 γ -PDT agents for targeting and photokilling against both Gram-positive and Gram-negative bacterial species [8,9]. These reported findings revealed the importance of cell surface interactions between multicationic drug molecules and anionic peptide residues in the cell wall. Specifically, several factors including differences in physiology, cell wall, and cytoplasmic membrane structures between Gram-positive and Gram-negative bacteria [10–12] affect the properties of particular functional groups to be attached on the fullerene cage to allow effective targeting selectivity, drug-delivery, and photodynamic inactivation.

Accordingly, we considered the structural modification of C_{60} to allow the increase of its solubility in physiologic media and its compatibility in an environment of bacterial disease in tissue. This led to our design and synthesis of [60]fullereryl monoadducts bearing a well-defined high number of cationic charges that hitherto had remained challenging and has rarely been reported to date. In this paper, we describe a rational linkage of water-soluble quaternary alkylammonium multi-salts and ester-amide functional groups to a well-defined pentacationic arm together with an efficient synthetic method for its attachment on a C_{60} nanocage. The synthesis led to the preparation of new pentacationic [60]fullerene-based nano-photosensitizers **1** and **2**, as shown in Schemes 1 and 2.

Scheme 1. The first synthetic steps of $C_{60}(>>ME_1N_6^+C_3)$ **1** and $C_{60}(>>ME_3N_6^+C_3)$ **2**.

Reagents and conditions: i. Meldrum's acid, 95 °C, 12 h; ii. NHS, DCC, THF, r.t., 1.0 day; iii. THF, r.t., 12 h; iv. CH_3-I , $CHCl_3-DMF$, 45 °C, 3.0 days; v. C_{60} , CBr_4 , DBU, toluene-DMF, r.t., 10 h.

Scheme 2. The second synthetic steps of $C_{60}(>>ME_1N_6^+C_3)$ **1** and $C_{60}(>>ME_3N_6^+C_3)$ **2**.

Reagents and conditions: vi. DCC, 2-methyl-2-propanol, CH_2Cl_2 , r.t., 8.0 h; vii. C_{60} , DBU, toluene-ODCB, r.t., 12 h; viii. TFA, CH_2Cl_2 , r.t., 15 h; ix. (a) $SOCl_2$, THF, reflux, 2.0 h; (b) $N_5C_3-NH_2$, 0 °C-r.t., 3.0 h; x. (a) aq. K_2CO_3 (10%); (b) CH_3-I , $CHCl_3-DMF$, 45 °C, 3.0 days.

In these structures, arm moieties each bearing a high number of cationic charges and an amide moiety are capable of inducing the H-bonding in the vicinity of the fullerene cage.

2. Results and Discussion

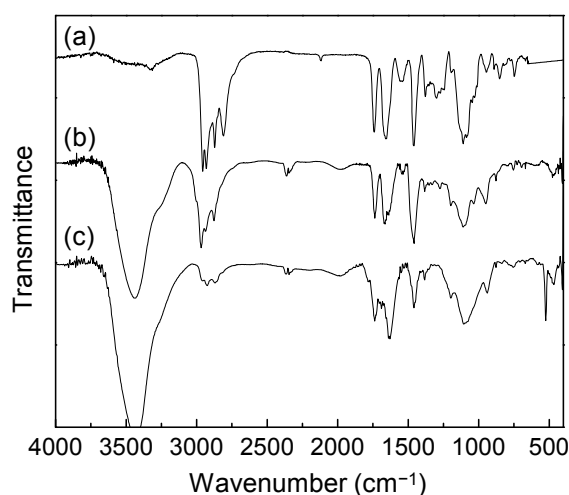
Enhancement of the hydrophilicity of fullerene derivatives can be achieved by incorporation of the oligo(ethylene glycol) unit [13–15], an aminoacid moiety [16], or ionic functional groups [17–19] as addend attachments of C_{60} cage. The resulting amphiphilic derivatives have been reported to undergo different forms of solid aggregation in aqueous solution if the hydrophilic moiety of the addend is insufficiently large to overcome the high hydrophobicity of the fullerene cage. To circumvent this solid aggregation problem, we undertook the effort to synthesize a well-defined water-soluble pentacationic N,N',N,N,N,N -hexapropyl-hexa(aminoethyl)amine arm moiety $C_3N_6^+$, as a charged C_3N_6 (**6**), with the number of charge being fixed at five per arm and used as the common synthon in the preparation of [60]fullerene monoadducts, as shown in Scheme 1. One example was given by the combination of a water-compatible ethylene glycol unit with a $C_3N_6^+$ arm to a single addend, such as the arm precursors $ME_1N_6^+C_3$ (**9**) and $ME_3N_6^+C_3$ (**10**), to enhance the water-solubility. Synthesis of **9** and **10** began with the reaction of either 2-methoxyethanol or triethylene glycol monomethyl ether with 2,2-dimethyl-1,3-dioxane-4,6-dione (**3**, Meldrum's acid) at 90–95 °C for a period of 12 h to afford malonic acid methoxyethyleneglycol ester, ME_1 (**4**), or malonic acid methoxytriethyleneglycol ester, ME_3 (**5**), in 95 or 90% yield, respectively. Amidation reaction of **4** and **5** was carried out by the treatment with N -hydroxysuccinamide and N,N' -dicyclohexyl carbodiimide (DCC) in anhydrous THF at ambient temperature over a period of 12 h, followed by the removal of insoluble byproduct of N,N' -dicyclohexyl urea and the further treatment with N,N',N,N,N,N -hexapropyl-hexa(aminoethyl)amine, C_3N_6 (**6**), for an additional period of 12 h. These reactions resulted in the corresponding products of methoxyethyleneglycol- $[N,N',N,N,N,N$ -hexapropyl-hexa(aminoethyl)-amino]malonamide ester, $ME_1N_6C_3$ (**7**), and methoxy-tri(ethyleneglycol)- $[N,N',N,N,N,N$ -hexapropyl-hexa(aminoethyl)amino]malonamide ester, $ME_3N_6C_3$ (**8**), in 82 and 80% yield, respectively. Quaternization reaction of mono- and tri(ethoxylated) hexaaminomalonamide precursors **7** and **8** using methyl iodide as the methylation agent at 45–50 °C for a period of 3.0 days afforded the corresponding methoxyethyleneglycol- $[N,N',N,N,N,N$ -hexapropyl-hexa(aminoethyl)amino]malonamide ester methyl quaternary ammonium salt, $ME_1N_6^+C_3$ (**9**), and its tri(ethyleneglycolated) analogue $ME_3N_6^+C_3$ (**10**) in 94 and 92% yield, respectively. Attachment of a C_{60} cage on the quaternary ammonium salts of malonamide was accomplished by the treatment of **9** and **10** in DMF with predissolved C_{60} in toluene in the presence of 1.8-diazabicyclo[5.4.0]-undec-7-ene (DBU) at ambient temperature for a period of 8.0 h. In this reaction, carbon tetrabromide was applied as the bromination agent for the replacement of malonyl α -proton *in situ*. To minimize the possible formation of partial fulleranyl byproducts containing multiaddends, an excess amount (2.5 equiv.) of C_{60} was applied. At the end of fullerenation, an excessive amount of C_{60} molecules was recovered and removed by repeatedly washing the crude products with toluene until the observation of a clear toluene solution in washings. The reaction procedure led to the isolation of methoxyethyleneglycol-(20-oxo-4,7,10,13,16-pentapropyl-4,7,10,13,16,19-hexaaza-nonadecan-19-yl)[60]fulleranyl malonate quaternary methyl ammonium salt,

$C_{60}(>>ME_1N_6^+C_3)$ (**1**), and its tri(ethyleneglycolated) analogue $C_{60}(>>ME_3N_6^+C_3)$ (**2**) in 55 and 50% yield, respectively.

Alternatively, as shown in Scheme 2, (ethyleneglycolated)malonic acids **4** and **5** were converted to their corresponding *tert*-butyl(2-methoxyethyl)malonate ester, $ME_1(t-C_4)$ (**11**), and its tri(ethyleneglycolated) analogue $ME_3(t-C_4)$ (**12**) in 74 and 77% yield, respectively, by an esterification reaction with *t*-butyl alcohol in anhydrous dichloromethane (DCM) in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) at ambient temperature for a period of 8.0 h. Subsequent fullereryl cyclopropanation reaction of **11** and **12** was carried out by treatment with [60]fullerene solution in a mixture of toluene and 1,2-dichlorobenzene in the presence of carbon tetrabromide and 1.8-diazabicyclo[5.4.0]-undec-7-ene (DBU) over a period of 12 h at room temperature. The products *tert*-butyl(2-methoxyethyl)[60]fullereryl malonate, $C_{60}[>ME_1(t-C_4)]$ (**13**), and *tert*-butyl(methoxytriethyleneglycol)[60]fullereryl malonate, $C_{60}[>ME_3(t-C_4)]$ (**14**), were purified by column chromatography [SiO_2 , toluene–ethyl acetate (20:1) as eluent] as brown solids in 78 and 75% yield, respectively. Direct transamidation reaction of **13** and **14** with C_3N_6 was not successful owing to the complicated side-reaction arising from the attack of C_3N_6 on C_{60} itself at elevated temperatures which is required for the effective transamidation. Therefore, we carried out the transformation of **13** and **14** to the corresponding methoxyethyleneglycol-(20-oxo-4,7,10,13,16-pentapropyl-4,7,10,13,16,19-hexaaza-nonadecan-19-yl)[60]fullereryl malonate **17**, $C_{60}(>>ME_1N_6C_3)$, and its methoxytriethyleneglycol ester analogous **18**, $C_{60}(>>ME_3N_6C_3)$, respectively, by the acid hydrolysis of **13** and **14** first to 2-[60]fullereryl-3-(2-methoxyethoxy)-3-oxopropanoic acid **15**, $C_{60}(>>ME_1H)$, and its methoxytriethyleneglycol ester analogous **16**, $C_{60}(>>ME_3H)$, respectively. The latter products were obtained as brown solids in yields of 89% and 87%, respectively. Conversion of **15** and **16** to their acid chloride intermediates was carried out by the reaction with thionyl chloride in THF. To minimize the amination of C_3N_6 on C_{60} , subsequent reaction with the acid chloride intermediate was performed at 0 °C to room temperature over a period of 3.0 h. A relatively pure $C_{60}(>>ME_1N_6C_3)$ **17** was obtained in 47% yield. It was accompanied with a small quantity of byproduct as a decarboxylated fullerene malonate-monoadduct that is removable. The stability of **17** and **18** in either solution or solid phase was found to be low owing to possible intermolecular complex formation between the hexamine moiety and C_{60} cage giving insoluble aggregate solids. Therefore, compound **17** and **18** was quaternized to the protonated ammonium–trifluoroacetate salt $C_{60}(>>ME_1N_6^+C_3)$ (**1'**) and $C_{60}(>>ME_3N_6^+C_3)$ (**2'**), respectively, with trifluoroacetic acid in a short period of time immediately after the workup procedure and purification. Final synthesis of the products pentacationic methoxyethyleneglycol-(20-oxo-4,7,10,13,16-pentapropyl-4,7,10,13,16,19-hexaaza-nonadecan-19-yl)[60]fullereryl malonate quaternary methyl ammonium salt $C_{60}(>>ME_1N_6^+C_3)$ (**1**), and its analogous methoxytriethyleneglycol ester $C_{60}(>>ME_3N_6^+C_3)$ (**2**), was carried out by the neutralization of **1'** and **2'** first with aqueous potassium carbonate in $CHCl_3$ as a biphasic solution. Resulting [60]fullereryl malonates **17** and **18**, respectively, were then transferred to a solvent mixture of anhydrous $CHCl_3$ and DMF (2:1) for methyl quaternization with an excess amount of iodomethane added portionwise over a period of 3.0 days at 45 °C to afford **1** and **2**. Successful amidation conversion from malonic acid **5** to $ME_3N_6C_3$ was verified by the FT-IR spectrum of **8** (Figure 1a) showing two clear and strong vibrational carbonyl absorption bands centered at 1667 and 1736 cm^{-1} corresponding to malonylamide carbonyl [$-NH-C(=O)-$] and ester carbonyl ($-C=O$) moieties, respectively. In the same spectrum, IR peaks located at 2952, 2928, 2862, and 2805 cm^{-1}

were assigned to the stretching absorption bands of aliphatic $-C-H$. Anti-symmetric deformations of $-CH_3$ groups and scissor vibrations of $-CH_2$ groups appeared as medium intensity bands centered around 1456 cm^{-1} , while symmetric deformations of CH_3 groups exhibited the absorption around 1379 cm^{-1} that overlaps with the $-N-C-$ stretching vibration of amide $[-N-(C=O)-]$ bands at 1400 cm^{-1} . A strong broad band centered at 1104 cm^{-1} was assigned to the stretching vibrations of $-C-O-C-$ and $-C-N-$ moieties. Methyl quaternization of **8**, having tertiary penta(ethylamino) arms, to $ME_3N_6^+C_3$ **10** with iodide salts showed retention of some of IR absorption bands (Figure 1b), except the shift of $-NH-C(=O)-$ band to 1669 cm^{-1} and a new broad band at 947 cm^{-1} , corresponding to the absorption of a number of quaternary $-C-N^+-$ bonds. Formation of $ME_3N_6^+C_3$ -attached C_{60} monoadduct was verified by the observation of a sharp characteristic fullerene half-cage absorption band of $C_{60}(>>ME_3N_6^+C_3)$ **2** at 524 cm^{-1} in the spectrum of Figure 1c. These results provided a good agreement of the product structure of **2**. Similar infrared absorption bands of $C_{60}(>>ME_1N_6^+C_3)$ **1** were observed, except a relatively smaller $-C-O-C-$ band than that of **2** centered at $1000\text{--}1200\text{ cm}^{-1}$.

Figure 1. FT-IR spectra of (a) $ME_3N_6C_3$ **8**, (b) $ME_3N_6^+C_3$ **10**, and (c) $C_{60}(>>ME_3N_6^+C_3)$ **2**.



Mass spectroscopic data collection of both **1** and **2** was proven to be difficult due to their polycationic nature and facile fragmentations occurring at the conjunction of the C_{60} cage and the pentacationic malonate arm, giving mainly the highly detectable C_{60} ion mass fragment at m/z 721, as displayed in MALDI-TOF mass spectra using sinapic acid as the matrix. All fragmented high mass ions were very weak in intensity including peaks at m/z 1930 (M^+-I^-) (supporting information) and 2019 (MH^+-I^-) as the molecular ion of **1** and **2**, respectively. The most pronounced mass fragmentation ions at m/z 874 and 876 in both spectra of **1** and **2** were assigned to the substructure of $C_{60}[>H(C=O)NHCH_2CH_2N^+-PrMe_2]$, indicating a main malonylamide moiety being attached on a C_{60} cage, consistent with the [60]fullerenyl monoadduct structure.

In the case 1H -NMR spectroscopic analyses, we first well-characterized the arm structures $ME_1(t-C_4)$ (**11**, Figure 2a) and $ME_3(t-C_4)$ (**12**) by identification of all protons (H_a , H_e , H_f , H_g , and H_i indicated in Scheme 2) with their assignments to peaks shown in the spectra. Attachment of **11** on C_{60} leading to a monoadduct $C_{60}[>ME_1(t-C_4)]$ (**13**) was evident by the disappearance of a H_a proton peak with the chemical shift at δ 3.33. It was also accompanied with up-fielded shifts of two proton peaks to δ 3.06 and 3.26, corresponding to the chemical shift of terminal methoxy protons H_g and ethylene oxide

protons H_f , in toluene- d_8 from δ 3.34 and 3.56 of **11** in $CDCl_3$, respectively, reflecting partly the solvent effect. Hydrolysis of **13** to $C_{60}(>>ME_1H)$ (**15**) was also apparent by the loss of *t*-butyl protons at δ 1.55, as shown in Figures 2b and 2c, accompanied with down-fielded shifts of proton peaks (H_g , H_f , and H_e) to δ 3.34, 3.71, and 4.59, respectively, in THF- d_8 showing a larger solvent effect. Monoadduct structures of **13** and **14** were also confirmed by the detection of well-defined characteristic fullerenyl sp^2 carbon peaks in their ^{13}C -NMR spectra (Figure 3b) showing a group of 26 and 27 peaks, respectively, each accounted for two carbons and a group of 6 single carbon peaks for **13** (two of these single carbon peaks may be derived from the slightly unsymmetrical environment around the malonate moiety) and 4 single carbon peaks for **14** in the region of δ 135–150, consistent with a C_v molecular symmetry for both **13** and **14**. It also displayed two clear carbonyl carbon peaks at δ 163.45 (O=C-O-*tert*-butyl) and 161.86 (O=C-O-) in toluene- d_8 , for **13** and the corresponding peaks at δ 163.95 and 162.16 in $CDCl_3$ for **14**. Evidence of the acid formation in the structure of **15** and $C_{60}(>>ME_3H)$ (**16**) was given by the solubility change and up-fielded shifts of the carbonyl chemical-shift to δ 161.13 (O=C-OH) and 161.62 (O=C-O-) in THF- d_8 - CS_2 (2:1) for **15** (Figure 3c), partly due to the solvent effect. More clear changes of carbonyl carbon peak positions were observed for the case of **16** showing down-fielded shifts to δ 163.79 (O=C-OH) and 164.12 (O=C-O-) (Figure 3d) from those of **14** in the same solvent, consistent with the corresponding structural modification.

Figure 2. 1H NMR spectra of (a) **11** in $CDCl_3$, (b) **13** in toluene- d_8 , (c) **15** in THF- d_8 , (d) **1'** in $CDCl_3$ -toluene- d_8 , and (e) **1** in DMSO- d_6 -toluene- d_8 .

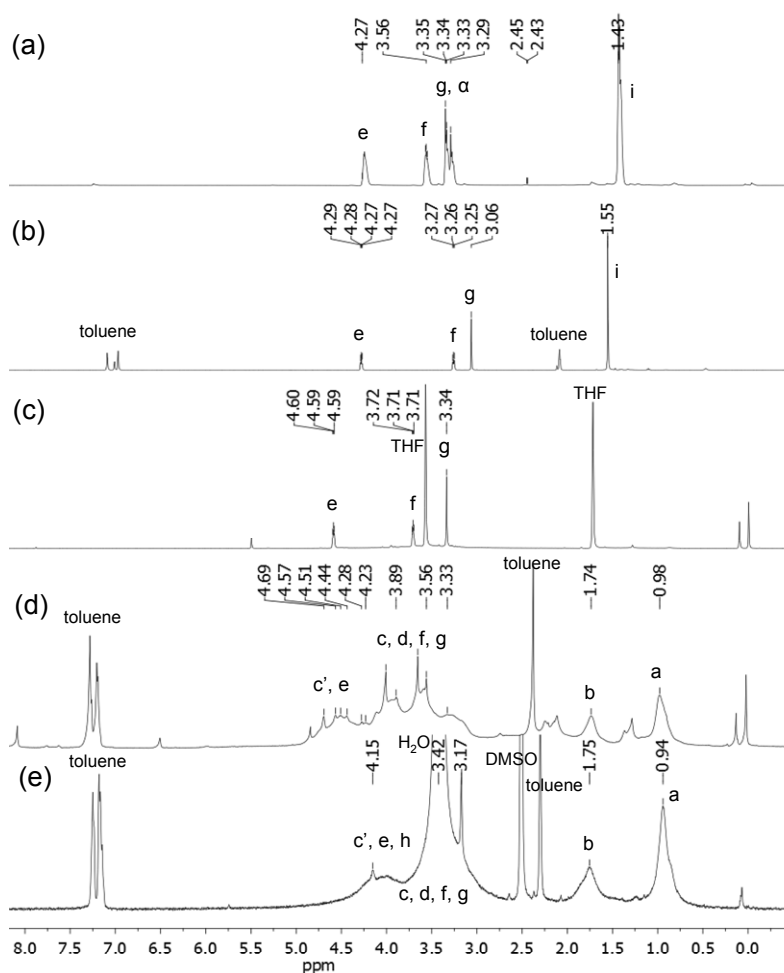
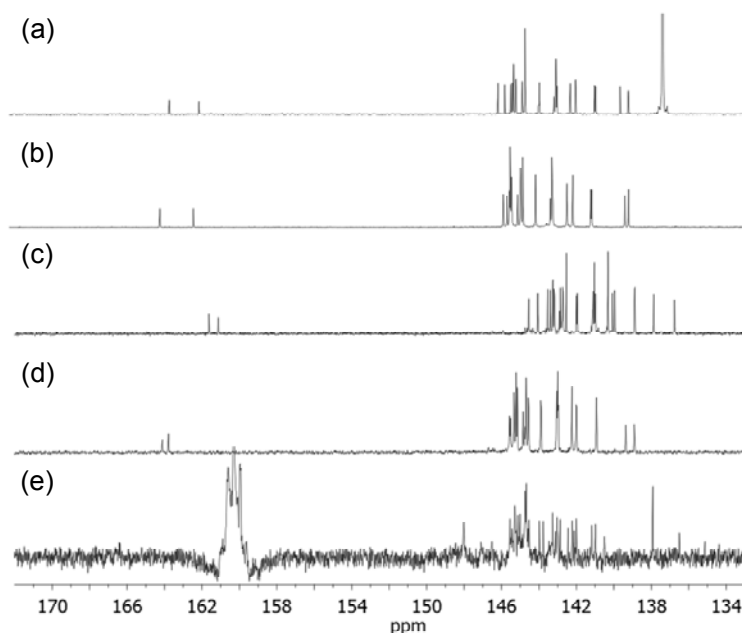


Figure 3. ^{13}C -NMR spectra of (a) **13** in toluene- d_8 ; (b) **14** in CDCl_3 ; (c) **15** in THF- d_8 - CS_2 ; (d) **16** in CDCl_3 ; and (e) **1'** in CDCl_3 .



In addition, the spectra of both compounds **15** and **16** displayed a group of 27 peaks each accounted for two sp^2 fullereryl carbons and four single sp^2 carbon peaks in the region of δ 135–145 provided the further confirmation of a C_v molecular symmetry of $\text{C}_{60}(>\text{ME}_1\text{H})$ and $\text{C}_{60}(>\text{ME}_3\text{H})$, respectively, as monoadducts without change during the chemical reaction.

Attachment of hexa(aminoethyl)amine C_3N_6^+ arm to the malonic acid moiety of $\text{C}_{60}(>\text{ME}_1\text{H})$ leading to the structure of $\text{C}_{60}(>\text{ME}_1\text{N}_6^+\text{C}_3)$ **1'** and subsequently **1** was confirmed by the analyses of both infrared and ^1H -NMR spectra. The latter of **1'** showed proton peaks corresponding to the C_3N_6^+ arm moiety with overlap of a number of terminal ammonium-propyl α -protons (H_c), α -protons to amide group (H_d), glycol ether protons (H_f), and terminal methoxy protons (H_g) giving broad multiplet peaks in the region of δ 3.0–4.25 in CDCl_3 –toluene- d_8 (Figure 2d). The second broad multiplet peaks in the region of δ 4.25–4.75 were assigned to ammonium-ethyl protons (H_c') and α -protons to the ester group (H_e). In the case of **1**, chemical shifts of similar groups of protons (H_c , H_d , H_f , and H_g) and (H_c' , H_e , and ammonium-methyl protons H_h) were assigned to those of broad multiplet peaks at δ 3.0–3.75 and 3.75–4.5, respectively, in $\text{DMSO-}d_6$ –toluene- d_8 (2:1), as shown in Figure 2(e). Interestingly, ^{13}C -NMR spectrum of $\text{C}_{60}(>\text{ME}_1\text{N}_6^+\text{C}_3)$ **1'** (Figure 3e) showed a group of 27 peaks each accounted for two sp^2 fullereryl carbons and four single sp^2 carbon peaks (two of them having an identical chemical shift) in the region of δ 135–150 that provided a clear confirmation of a C_v molecular symmetry of $\text{C}_{60}(>\text{ME}_1\text{N}_6^+\text{C}_3)$ monoadduct.

3. Experimental

3.1. Materials

The reagents 2-methoxyethanol, triethylene glycol monomethyl ether, 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid), *N*-hydroxysuccinamide (NHS), *N,N'*-dicyclohexyl carbodiimide (DCC),

iodomethane, carbon tetrabromide (CBr₄), 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU), 2-methyl-2-propanol, trifluoroacetic acid (TFA), thionyl chloride (SOCl₂), K₂CO₃, γ -butyrolactone (GBL), BF₃·Et₂O, triethylamine, and pyridine were purchased from Aldrich Chemicals and used without further purification. Malonyl chloride was purchased from TCI America. A C₆₀ sample with a purity of 99.0% was used. Sodium sulfate was employed as a drying agent. Solvents were routinely distilled before use.

3.2. Spectroscopic Measurements

Infrared spectra were recorded as KBr pellets on a Thermo Nicolet Avatar 370 FT-IR spectrometer. ¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker Avance Spectrospin-500 spectrometer. UV-Vis spectra were recorded on a Perkin Elmer Lambda 750 UV-Vis-NIR Spectrometer. MALDI-mass spectra were recorded on a WATERS Micromass MALDI-TOF mass spectrometer. Elemental analysis was taken by Galbraith Laboratories, Inc. (Knoxville, TN, USA).

3.3. Synthetic Procedures

3.3.1. Synthesis of Malonic Acid Methoxyethyleneglycol Ester, ME₁ (**4**)

A mixture of 2-methoxyethanol (1.0 g, 13.1 mmol) and 2,2-dimethyl-1,3-dioxane-4,6-dione (**3**, Meldrum's acid, 1.9 g, 13.2 mmol) was stirred under an Ar atmosphere over a period of 12 h at 95 °C. The reaction mixture was cooled to room temperature, treated with aqueous sodium carbonate solution (5%), and washed with diethyl ether. The resulting aqueous layer was subsequently treated with dil. HCl (2.0 N) and extracted with ethyl acetate (50 mL). The ethyl acetate solution was dried over Na₂SO₄ and concentrated on rotary evaporator to give the product ME₁ (**4**) in 95% yield (2.0 g). Spectroscopic data: FT-IR (KBr) ν_{\max} 3060 (w), 2880 (w), 2814 (w), 1727 (vs), 1458 (w), 1399 (m), 1318 (s), 1249 (s), 1134 (s), 1100 (s), 1039 (m), 952 (w), 848 (s), and 755 (m) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃, ppm) δ 10.60 (s, br, 1H), 4.31 (t, *J* = 4.63 Hz, 2H), 3.62 (t, *J* = 4.63 Hz, 2H), 3.45 (s, 2H), and 3.38 (s, 3H).

3.3.2. Synthesis of Malonic Acid Methoxytriethyleneglycol Ester, ME₃ (**5**)

A mixture of triethylene glycol monomethyl ether (1.0 g, 6.1 mmol) and 2,2-dimethyl-1,3-dioxane-4,6-dione (**3**, Meldrum's acid, 0.9 g, 6.2 mmol) was stirred under Ar atmosphere over a period of 12 h at 90 °C. The reaction mixture was cooled to room temperature, treated with aqueous sodium carbonate solution (5%), and washed with diethyl ether. The resulting aqueous layer was subsequently treated with dil. HCl (2.0 N) and extracted with ethyl acetate (50 mL). The ethyl acetate solution was dried over Na₂SO₄ and concentrated on rotary evaporator to give the product ME₃ (**5**) in 90% yield (1.37 g). Spectroscopic data: FT-IR (KBr) ν_{\max} 3056 (w), 2880 (w), 2817 (w), 1728 (vs), 1455 (w), 1394 (m), 1318 (s), 1251 (s), 1134 (vs), 1098 (vs), 1034 (m), 952 (w), 847 (s), and 753 (m) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃, ppm) δ 9.65 (s, br, 1H), 4.32 (t, *J* = 4.50 Hz, 2H), 3.70 (t, *J* = 4.50 Hz, 2H), 3.65–3.55 (m, 8H), 3.42 (s, 3H), and 3.41 (s, 2H).

3.3.3. Synthesis of Methoxyethyleneglycol-[*N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amino]-malonamide Ester, ME₁N₆C₃ (**7**)

A mixture of malonic acid methoxyethyleneglycol ester **4** (0.5 g, 3.08 mmol), *N*-hydroxysuccinamide (0.35 g, 3.08 mmol), and *N,N'*-dicyclohexyl carbodiimide (DCC, 0.63 g, 4.0 mmol) in anhydrous tetrahydrofuran (20 mL) were stirred under N₂ atmosphere over a period of 12 h at ambient temperature. The resulting white solids of *N,N'*-dicyclohexyl urea byproduct were filtered off and the filtrate was taken into a second round-bottom flask containing *N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amine **6** (1.49 g, 3.08 mmol). The mixture was stirred under N₂ atmosphere for an additional period of 12 h. At the end of the reaction, the solvent was removed on rotavap. To this residue, ice-cold hexane–dichloromethane (1:1, 15 mL) was added followed by filtration to remove further white solids of *N*-hydroxysuccinamide. The filtrate was washed with aqueous sodium carbonate (5%) solution (10 mL). The organic layer was then dried and concentrated to give ME₁N₆C₃ (**7**) as yellow liquid in 82% yield (1.59 g). Spectroscopic data: ESI–MS (rel. intensity) *m/z* 568, 570 (M–CH₃OCH₂CH₂, 75%), 571, 629 (M⁺), 630 (MH⁺, 100%), 631, 646, 666, 670, 688, 700, 715 [MH⁺+CH₂CH₂N(CH₂CH₂CH₃), 48%], 716, 755, 757, 772, 773, 775, 800 (MH+2[CH₂CH₂N(CH₂CH₂CH₃)], 15%), 801, 832, 860, 1150, 1210, 1212 (the dimer mass–CH₃OCH₂), 1236, and 1252 (from the dimer ion mass); FT-IR (KBr) ν_{\max} 3259 (w), 2956 (vs), 2932 (s), 2872 (m), 2808 (m), 1742 (s), 1660 (s), 1550 (w), 1459 (s), 1273 (m), 1128 (m), 1074 (s), 1031 (m), and 742 (w) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃, ppm) δ 4.29 (t, *J* = 4.25 Hz, 2H), 3.61 (t, *J* = 4.25 Hz, 2H), 3.40 (s, 3H), 3.34–3.32 (m, 4H), 2.38–2.57 (m, 30H), 1.47–1.44 (m, 12H), and 0.90–0.87 (t, *J* = 7.15 Hz, 18H); ¹³C-NMR (500 MHz, CDCl₃, ppm) δ 168.78 (–C=O), 164.65 (O=C–NH–), 70.06, 64.15, 58.90, 57.35, 57.29, 57.13, 56.83 (4C), 56.72, 56.55, 53.65, 53.00, 52.81, 52.58, 52.46, 52.00, 41.60, 37.58, 20.41, 20.39, 20.37, 20.32 (2C), 20.18, 11.90 (3C), 11.87 (2C), and 11.74.

3.3.4. Synthesis of Methoxy-tri(ethyleneglycol)-[*N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)-amino]-malonamide Ester, ME₃N₆C₃ (**8**)

A mixture of malonic acid methoxytriethyleneglycol ester **5** (1.0 g, 4.0 mmol), *N*-hydroxysuccinamide (0.45 g, 4.0 mmol), and *N,N'*-dicyclohexyl carbodiimide (0.82 g, 4.0 mmol) in anhydrous tetrahydrofuran (30 mL) were stirred under N₂ atmosphere for a period of 12 h at ambient temperature. The resulting white solids of *N,N'*-dicyclohexyl urea byproduct were filtered off and the filtrate was taken into a second round-bottom flask containing *N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amine **6** (1.94 g, 3.9 mmol). The mixture was stirred under N₂ atmosphere for an additional period of 12 h. At the end of the reaction, the solvent was removed on rotavap. To this residue, ice-cold hexane–dichloromethane (1:1, 20 mL) was added followed by filtration to remove white solids of *N*-hydroxysuccinamide. The filtrate was washed with aqueous sodium carbonate (5%) solution (10 mL). The organic phase was then dried and concentrated to give ME₃N₆C₃ (**8**) as yellow liquid in 80% yield (2.29 g). Spectroscopic data: ESI–MS (rel. intensity) *m/z* 512, 568 [M–CH₃O(CH₂CH₂O)₂CH₂, 65%], 660 (M–CH₃OCH₂CH₂, 55%), 716, 718 (MH⁺, 100%), 734, 776, 803 [MH⁺+CH₂CH₂N(CH₂CH₂CH₃) from the dimer mass, 46%], 819, 860, 888 (MH⁺+2[CH₂CH₂N(CH₂CH₂CH₃)] from the dimer mass, 8%), 944, 951, 1008, 1090, 1226 (the dimer

mass-[MeO(EG)₃-CO], 3%), 1238, 1282, 1340, 1430, and 1434 (the dimer ion); FT-IR (KBr) ν_{\max} 3328 (w), 2952 (m), 2928 (m), 2862 (m), 2805 (m), 1736 (vs), 1667 (s), 1533 (m), 1456 (m), 1400 (m), 1379 (m), 1245 (m), 1104 (vs), 1032 (w), 938 (w), and 732 (m) cm^{-1} ; ¹H-NMR (500 MHz, CDCl₃, ppm) δ 4.22 (t, J = 4.68 Hz, 2H), 3.65 (t, J = 4.68 Hz, 2H), 3.58–3.60 (m, 6H), 3.48 (t, J = 4.60 Hz, 2H), 3.32 (s, 5H), 3.26 (s, br, 2H), 2.31–2.48 (m, 30H), 1.40–1.38 (m, 12H), and 0.82–0.79 (t, J = 6.95 Hz, 18H); ¹³C-NMR (500 MHz, CDCl₃, ppm) δ 168.83 (–C=O), 164.70 (O=C–NH–), 71.86 (2C), 70.53, 70.51, 68.74, 64.30, 58.95, 57.32 (2C), 57.14, 56.83 (4C), 56.72, 56.53, 53.64, 53.41, 52.98 (2C), 52.80, 52.45, 41.54, 37.55, 20.40, 20.37, 20.31 (3C), 20.24, 11.91 (3C), 11.88 (2C), and 11.76.

3.3.5. Synthesis of Methoxyethyleneglycol-[*N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amino]-malonamide Ester Quaternary Methyl Ammonium Salt, ME₁N₆⁺C₃ (**9**)

A solution of malonamide ester **7** (1.0 g, 1.6 mmol) in anhydrous chloroform–DMF (10:1) was added iodomethane (6.0 mL, excess, in portions) and stirred at 45 °C for a period of 3.0 days. At the end of quaternization, the solvent was evaporated to afford ME₁N₆⁺C₃ (**9**) in 94% yield (2.0 g). Spectroscopic data: FT-IR (KBr) ν_{\max} 3444 (s), 3236 (m), 2968 (vs), 2937 (vs), 2877 (m), 1735 (s), 1667 (s), 1536 (m), 1459 (vs), 1331 (w), 1126 (m), 1032 (m), 948 (m), 872 (w), 750 (vs), and 661 (m) cm^{-1} ; ¹H-NMR (500 MHz, DMSO-*d*₆, ppm) δ 3.80–4.25 (m, br, 16H), 3.22–3.80 (m, br, 41H), 1.50–1.85 (m, br, 12H), and 0.88–0.99 (m, br, 18H); ¹³C-NMR (500 MHz, DMSO-*d*₆, ppm) δ 168.09 (–C=O), 166.69 (O=C–NH–), 79.90, 79.70, 70.01, 64.28 (4C), 63.59 (5C), 58.54, 55.54 (4C), 53.96 (2C), 49.37 (3C), 48.85 (2C), 42.62, 16.27 (2C), 15.95 (4C), 10.94 (2C), 10.82 (2C), and 10.72 (2C) (one peak is covered by DMSO peaks). Anal. Calcd for C₃₄H₇₂N₆O₄·4.5CH₃I·2H₂O (based on 90% quaternization on average): C, 35.47; H, 6.92; N, 6.45; I, 43.80; O, 7.36%. Found: C, 34.72; H, 6.82; N, 6.46; I, 42.31%.

3.3.6. Synthesis of Methoxy-tri(ethyleneglycol)-[*N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)-amino]-malonamide Ester Quaternary Methyl Ammonium Salt, ME₃N₆⁺C₃ (**10**)

A solution of malonamide ester **8** (0.50 g, 0.70 mmol) in anhydrous chloroform–DMF (10:1) was added iodomethane (3.0 mL, excess, in portions) and stirred at 45 °C for a period of 3.0 days. At the end of quaternization, the solvent was evaporated to afford ME₃N₆⁺C₃ (**10**) in 92% yield (0.92 g). Spectroscopic data: ESI–MS (rel. intensity) m/z 448 (100%), 503, 588 (40%), 731 (M⁺–5I–4CH₃, 6%), 760, 815, 872, 873 (M⁺–4I–3CH₃, 8%), 930, 1014, 1016 (M⁺–3I–2CH₃, 13%), 1074, 1099, 1157 (M⁺–2I–CH₃, 37%), 1198, 1216, 1299, 1300 (M⁺–I, 22%), 1385, 1391, 1392, 1442, 1443, and 1444 (M⁺+H₂O); FT-IR (KBr) ν_{\max} 3436 (vs), 3255 (m), 3006 (m), 2968 (s), 2935 (s), 2877 (m), 1736 (s), 1665 (s), 1634 (s), 1545 (w), 1459 (s), 1383 (w), 1351 (w), 1332 (w), 1271 (w), 1200 (m), 1110 (s), 1034 (m), 950 (m), 877 (w), 757 (w), and 600 (m) cm^{-1} ; ¹H-NMR (500 MHz, DMSO-*d*₆, ppm) δ 3.80–4.25 (m, br, 16H), 2.90–3.80 (m, br, 49H), 1.50–1.80 (m, br, 12H), and 0.85–0.99 (m, br, 18H); ¹³C-NMR (500 MHz, DMSO-*d*₆, ppm) δ 168.13 (–C=O), 166.69 (O=C–NH–), 71.72, 70.23, 70.18, 70.03, 68.57, 63.56 (9C), 54.79 (6C), 49.05 (5C), 42.62, 16.18 (2C), 15.97 (4C), 10.95 (2C), 10.82 (3C), and 10.70 (one peak is covered by DMSO peaks). Anal. Calcd for C₃₈H₈₀N₆O₆·4CH₃I·3H₂O (based on 80% quaternization on average): C, 37.67; H, 7.38; N, 6.28; I, 37.91; O, 10.76%. Found: C, 36.10; H, 7.03; N, 6.81; I, 37.90%.

3.3.7. Synthesis of Pentacationic Methoxyethyleneglycol-(20-oxo-4,7,10,13,16-pentapropyl-4,7,10,13,16,19-hexaaza-nonadecan-19-yl)[60]fullerenyl Malonate Quaternary Methyl Ammonium Salt, $C_{60}(>>ME_1N_6^+C_3)$ (**1**)

Finely divided [60]fullerene (0.94 g, 1.30 mmol, more than two-fold excess to allow the formation of monoadduct only) was taken into a round bottom flask and added anhydrous toluene (700 mL) under nitrogen. The solution was stirred for 12 h at ambient temperature to ensure complete dissolution of C_{60} . To the resulting purple-colored solution added carbon tetrabromide (0.19 g, 0.57 mmol) followed by a solution of the compound **9** (0.70 g, 0.52 mmol) in anhydrous DMF (100 mL). The solution mixture was stirred for an additional 30 min and added slowly 1.8-diazabicyclo[5.4.0]-undec-7-ene (DBU, 0.17 g, 1.15 mmol) over a period of 45 min. The color of solution slowly turns into brown in a reaction period of 8.0 h. The solution was then concentrated on rotavap to roughly 100 mL. Upon the addition of methanol to this concentrated solution, the crude product was precipitated as brown solids which were collected via centrifugation. Unreacted C_{60} in the crude solids was removed by repeated washings with toluene (5×100 mL) until no color in the washing solution or filtrate. The remaining product of $C_{60}(>>ME_1N_6^+C_3)$ (**1**) was obtained as brown solids in 55% yield (0.59 g, after recovered C_{60}). Spectroscopic data: FT-IR (KBr) ν_{\max} 3383 (vs), 2963 (m), 2932 (m), 2870 (m), 2814 (w), 1739 (s), 1686 (s), 1625 (s), 1455 (vs), 1426 (s), 1373 (w), 1240 (w), 1067 (s), 1031 (s), 947 (m), 728 (m), 575 (m), and 525 (vs, a characteristic band of C_{60} monoadduct) cm^{-1} ; UV-Vis (DMF, cutoff at 268 nm, 2.0×10^{-5} M) λ_{\max} 323 nm (shoulder peak); $^1\text{H-NMR}$ [500 MHz, $\text{DMSO-}d_6$ -toluene- d_8 (2:1), ppm] δ 3.80–4.25 (m, br, 16H), 2.90–3.80 (m, br, 39H), 1.50–1.80 (m, br, 12H), and 0.88–0.99 (m, br, 18H). We found that electronic interferences of iodide anions in a high quantity with the fullerene cage or possible partial electron-transfer events prohibited the detection of fullerenyl carbon peaks (in low signal intensity).

3.3.8. Synthesis of Pentacationic Methoxy-tri(ethyleneglycol)-(20-oxo-4,7,10,13,16-pentapropyl-4,7,10,13,16,19-hexaaza-nonadecan-19-yl)[60]fullerenyl Malonate Methyl Quaternary Ammonium Salt, $C_{60}(>>ME_3N_6^+C_3)$ (**2**)

Finely divided [60]fullerene (1.0 g, 1.40 mmol, more than two-fold excess to allow the formation of monoadduct only) was taken into a round bottom flask and added anhydrous toluene (700 mL) under nitrogen. The solution was stirred for 12 h at ambient temperature to ensure complete dissolution of C_{60} . To the resulting purple-colored solution added carbon tetrabromide (0.17 g, 0.51 mmol) followed by a solution of compound **10** (0.65 g, 0.45 mmol) in anhydrous DMF (100 mL). The solution mixture was stirred for an additional 30 min of stirring and added slowly 1.8-diazabicyclo[5.4.0]-undec-7-ene (DBU, 0.15 g, 0.98 mmol) over a period of 45 min. The color of solution slowly turns into brown in a reaction period of 8 h. The solution was then concentrated on rotavap to roughly 100 mL. Upon the addition of methanol to this concentrated solution, the crude product was precipitated as brown solids which were collected via centrifugation. Unreacted C_{60} in the crude solids was removed by repeated washings with toluene (5×100 mL) until no color in the washing solution or filtrate. The remaining product of $C_{60}(>>ME_3N_6^+C_3)$ (**2**) was obtained as brown solids in 50% yield (0.343 g, after recovered C_{60}). Spectroscopic data: FT-IR (KBr) ν_{\max} 3433 (vs), 3262 (s), 2963 (m), 2925 (m), 2868 (m), 2809

(w), 1736 (s), 1695 (m), 1630 (s), 1459 (s), 1384 (w), 1197 (m), 1107 (s), 1071 (s), 938 (m), 757 (w), and 525 (s, a characteristic band of C₆₀ monoadduct) cm⁻¹; UV-Vis (DMF, cutoff at 268 nm, 2.0 × 10⁻⁵ M) λ_{max} 323 nm (shoulder peak); ¹H-NMR [500 MHz, DMSO-*d*₆-toluene-*d*₈ (2:1), ppm] δ 3.80–4.20 (m, br, 16H), 2.90–3.80 (m, br, 47H), 1.50–1.75 (m, br, 12H), and 0.88–0.99 (m, br, 18H). We found that electronic interferences of iodide anions in a high quantity with the fullerene cage or possible partial electron-transfer events prohibited the detection of fullerene carbon peaks (in low signal intensity).

3.3.9. Synthesis of *tert*-Butyl(2-methoxyethyl)malonate, ME₁(*t*-C₄) (**11**)

A mixture of malonic acid methoxyethyleneglycol ester **4** (1.0 g, 6.16 mmol), 2-methyl-2-propanol (0.54 g, 7.39 mmol), and *N,N'*-dicyclohexyl carbodiimide (DCC, 1.27 g, 6.16 mmol) in anhydrous dichloromethane (20 mL) were stirred under atmospheric pressure of N₂ over a period of 8.0 h at ambient temperature. The resulting white solid of *N,N'*-dicyclohexyl urea was filtered and the filtrate was washed with aqueous sodium carbonate solution (5%, 10 mL). The organic layer was then dried over sodium sulfate and concentrated on rotavap to give ME₁(*t*-C₄) (**11**) in 74% yield (1.0 g) as light yellow liquid. Spectroscopic data: FT-IR (KBr) ν_{max} 2980 (w), 2930 (w), 2880 (w), 2824 (w), 1748 (s), 1726 (vs), 1455 (w), 1406 (w), 1393 (w), 1368 (m), 1330 (m), 1281 (m), 1250 (m), 1199 (m), 1127 (vs), 1100 (m), 1037 (s), 966 (m), 864 (m), 838 (m), 759 (w), and 738 (w) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃, ppm) δ 4.27 (t, *J* = 3.94 Hz, 2H), 3.56 (t, *J* = 3.94 Hz, 2H), 3.34 (s, 3H), 3.29 (s, 2H, H_α), and 1.43 (s, 9H).

3.3.10. Synthesis of *tert*-Butyl(methoxy-triethyleneglycol)malonate, ME₃(*t*-C₄) (**12**)

A mixture of malonic acid methoxytriethyleneglycol ester **5** (3 g, 11.98 mmol), 2-methyl-2-propanol (1.06 g, 14.38 mmol), and *N,N'*-dicyclohexyl carbodiimide (2.47 g, 11.98 mmol) in anhydrous dichloromethane (20 mL) were stirred under atmospheric pressure of N₂ over a period of 8.0 h at ambient temperature. The resulting white solid of *N,N'*-dicyclohexyl urea was filtered and the filtrate was washed with aqueous sodium carbonate solution (5%, 10 mL). The organic layer was then dried over sodium sulfate and concentrated on rotavap to give ME₃(*t*-C₄) (**12**) in 77% yield (2.85 g) as light yellow liquid. Spectroscopic data: FT-IR (KBr) ν_{max} 2973 (w), 2930 (w), 2876 (w), 2817 (w), 1748 (s), 1727 (vs), 1455 (w), 1393 (w), 1368 (m), 1330 (m), 1282 (m), 1250 (m), 1198 (m), 1134 (vs), 1104 (vs), 1040 (m), 966 (m), 864 (m), 759 (w), and 735 (w) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃, ppm) δ 4.26 (t, *J* = 4.50 Hz, 2H), 3.68 (t, *J* = 4.50 Hz, 2H), 3.63 (m, 6H), 3.52 (t, *J* = 4.50 Hz, 2H), 3.35 (s, 3H), 3.29 (s, 2H, α-H), and 1.44 (s, 9H).

3.3.11. Synthesis of *tert*-Butyl(2-methoxyethyl)[60]fullerene Malonate, C₆₀[>ME₁(*t*-C₄)] (**13**)

Finely divided [60]fullerene (1.23g, 1.70 mmol) was taken into a round bottom flask and added anhydrous toluene (850 mL) and 1,2-dichlorobenzene (30 mL) under nitrogen. The solution was stirred for 1.0 h at ambient temperature to ensure complete dissolution of C₆₀. To the resulting purple-colored solution was added carbon tetrabromide (0.50 g, 1.51 mmol) followed by a solution of *tert*-butyl(2-methoxyethyl)malonate (**11**, 0.30 g, 1.37 mmol) in anhydrous toluene (10 mL). The solution mixture was stirred for an additional 30 min and added slowly 1.8-diazabicyclo[5.4.0]undec-7-ene

(DBU, 0.44 g, 2.88 mmol) over a period of 1.0 h. The color of solution slowly turned into brown in a reaction period of 12 h. The solution was then concentrated on a rotavap. The resulting crude product was purified using column chromatography with silica gel as the stationary phase and toluene–ethyl acetate (20:1) as eluent, giving the isolation of *tert*-butyl(2-methoxyethyl)[60]fullerenyl malonate (**13**), C₆₀[>ME₁(*t*-C₄)], as brown solids in 78% yield (1.00 g). Spectroscopic data: FT-IR (KBr) ν_{\max} 3442 (br, s), 2972 (w), 2917 (w), 2873 (w), 2814 (w), 1739 (vs), 1645 (m), 1426 (m), 1390 (w), 1366 (m), 1268 (s), 1232 (vs), 1179 (m), 1151 (s), 1110 (m), 1059 (m), 1026 (m), 828 (w), 738 (w), 704 (w), 577 (m), 551 (m), and 525 (vs, a characteristic band of C₆₀ monoadduct) cm⁻¹; ¹H-NMR (500 MHz, toluene-*d*₈, ppm) δ 4.28 (t, *J* = 4.55 Hz, 2H), 3.26 (t, *J* = 4.55 Hz, 2H), 3.06 (s, 3H), and 1.55 (s, 9H); ¹³C-NMR (500 MHz, toluene-*d*₈, ppm) δ 163.45 (O=C-O-*tert*-butyl), 161.86 (O=C-O-), 145.89 (2C), 145.53 (2C), 145.22 (2C), 145.13 (2C), 145.06 (3C), 144.95 (2C), 144.93 (2C), 144.60 (2C), 144.59 (2C), 144.46 (3C), 144.45 (4C), 143.71 (2C), 143.68 (2C), 142.91 (C), 142.89 (C), 142.83 (2C), 142.81 (3C), 142.80 (3C), 142.74 (2C), 142.03 (2C), 142.02 (2C), 141.76 (2C), 141.74 (2C), 140.74 (2C), 140.68 (2C), 139.37 (2C), 138.93 (2C), 84.13, 72.07 (fullerenyl sp³ carbons, 2C), 69.74, 65.49, 58.00, 53.45, and 27.49 (3C).

3.3.12. Synthesis of *tert*-Butyl(methoxy-triethyleneglycol)[60]fullerenyl Malonate, C₆₀[>ME₃(*t*-C₄)] (**14**)

Finely divided [60]fullerene (1.86 g, 2.59 mmol) was taken into a round bottom flask and added anhydrous toluene (850 mL) and 1,2-dichlorobenzene (50 mL) under nitrogen. The solution was stirred for 1.0 h at ambient temperature to ensure complete dissolution of C₆₀. To the resulting purple-colored solution was added carbon tetrabromide (0.75 g, 2.27 mmol) followed by a solution of *tert*-butyl(methoxy-triethyleneglycol)malonate **12** (0.63 g, 2.07 mmol) in anhydrous toluene (10 mL). The solution mixture was stirred for an additional 30 min and added slowly 1.8-diazabicyclo[5.4.0]-undec-7-ene (DBU, 0.66 g, 4.35 mmol) over a period of 1.0 h. The color of solution slowly turned into brown in a reaction period of 12 h. The solution was then concentrated on rotavap and the resulting crude product was purified by column chromatography with silica gel as the stationary phase and toluene–ethyl acetate (9:1) as eluent to afford C₆₀[>ME₃(*t*-C₄)] (**14**) as brown solids in 75% yield (1.60 g). Spectroscopic data: FT-IR (KBr) ν_{\max} 3421 (br, s), 2967 (w), 2914 (w), 2864 (w), 2814 (w), 1740 (s), 1634 (m), 1456 (m), 1426 (m), 1392 (m), 1368 (m), 1269 (s), 1253 (s), 1226 (s), 1180 (m), 1154 (s), 1108 (m), 1029 (m), 845 (m), 737 (m), 702 (m), 669 (m), 576 (m), 550 (m), and 525 (vs) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃, ppm) δ 4.65 (t, *J* = 4.60 Hz, 2H), 3.90 (t, *J* = 4.60 Hz, 2H), 3.73 (t, *J* = 4.60 Hz, 2H), 3.65–3.72 (m, 4H), 3.56 (t, *J* = 4.60 Hz, 2H), 3.39 (s, 3H), and 1.70 (s, 9H); ¹³C-NMR (500 MHz, CDCl₃, ppm) δ 163.95 (O=C-O-*tert*-butyl), 162.16 (O=C-O-), 145.61 (2C), 145.41 (2C), 145.29 (2C), 145.25 (4C), 145.22 (2C), 145.16 (3C), 144.85 (2C), 144.71 (C), 144.69 (3C), 144.66 (2C), 144.57 (4C), 143.89 (3C), 143.09 (2C), 143.08 (2C), 143.01 (4C), 142.99 (2C), 142.97 (2C), 142.23 (2C), 142.21 (2C), 141.91 (2C), 141.90 (2C), 140.94 (2C), 140.89 (2C), 139.11 (2C), 138.92 (2C), 85.16, 71.95 (fullerenyl sp³ carbons, 2C), 71.84, 70.68, 70.67, 70.65, 68.84, 66.07, 59.10, 53.06, and 28.07 (3C).

3.3.13. Synthesis of 2-[60]Fullerenyl-3-(2-methoxyethoxy)-3-oxopropanoic Acid, C₆₀(>ME₁H) (**15**)

The compound of [60]fullerenyl malonate **13** (0.5 g, 0.64 mmol) was taken into a round bottom flask containing anhydrous dichloromethane (50 mL) and purged with N₂ for 15 minutes at ambient temperature. To this reaction mixture was added trifluoroacetic acid (30 mL, excess) and stirred for overnight at room temperature. At the end of the reaction, dichloromethane was removed on rotavap. Additional dichloromethane (3 × 15 mL) was added and removed on rotavap in order to fully eliminate an excessive amount of trifluoroacetic acid. The resulting residue was then washed with diethyl ether (2 × 15 mL) to afford C₆₀(>ME₁H) (**15**) as brown solids in 89% yield (0.50 g). Spectroscopic data: FT-IR (KBr) ν_{\max} 3670 (br, s), 2977 (w), 2917 (w), 2878 (w), 2814 (w), 1792 (w), 1750 (s), 1708 (s), 1681 (m), 1541 (w), 1427 (m), 1390 (w), 1366 (m), 1267 (s), 1250 (s), 1231 (s), 1186 (m), 1114 (w), 1059 (w), 1020 (w), 864 (w), 743 (w), 701 (m), 572 (m), 552 (m), and 525 (vs, a characteristic band of C₆₀ monoadduct) cm⁻¹; ¹H-NMR (500 MHz, THF-*d*₈, ppm) δ 4.59 (t, *J* = 4.50 Hz, 2H), 3.71 (t, *J* = 4.50 Hz, 2H), and 3.34 (s, 3H); ¹³C-NMR [500 MHz, THF-*d*₈-CS₂ (2:1), ppm] δ 161.62 (O=C-O-), 161.13 (O=C-OH), 144.54 (2C), 144.06 (2C), 143.53 (2C), 143.39 (2C), 143.26 (2C), 143.25 (2C), 143.19 (2C), 143.16 (2C), 142.91 (C), 142.85 (2C), 142.78 (C), 142.73 (2C), 142.70 (2C), 142.53 (4C), 141.99 (2C), 141.95 (2C), 141.14 (C), 141.12 (C), 141.10 (2C), 141.04 (4C), 140.98 (2C), 140.31 (4C), 140.07 (2C), 139.95 (2C), 138.89 (2C), 138.87 (2C), 137.87 (2C), 136.76 (2C), 70.48 (fullerenyl sp³ carbons, 2C), 68.14, 63.98, 56.30, and 51.46

3.3.14 Synthesis of 2-[60]Fullerenyl-3-(methoxy-triethyleneglycol)-3-oxopropanoic Acid, C₆₀(>ME₃H) (**16**)

The compound of [60]fullerenyl malonate **14** (0.75 g, 0.73 mmol) was taken into a round bottom flask containing anhydrous dichloromethane (50 mL) and purged with N₂ for a period of 15 minutes at ambient temperature. To this reaction mixture was added trifluoroacetic acid (50 mL) and stirred for overnight at room temperature. At the end of the reaction, dichloromethane was removed on rotavap. Additional dichloromethane (3 × 20 mL) was added and removed on rotavap in order to fully eliminate an excessive amount of trifluoroacetic acid. The resulting residue was then washed with diethyl ether (3 × 20 mL) to afford C₆₀(>ME₃H) (**16**) as brown solids in 87% yield (0.62 g). Spectroscopic data: FT-IR (KBr) ν_{\max} 3673 (br, s), 2917 (w), 2894 (w), 2870 (w), 2814 (w), 1788 (w), 1741 (vs), 1578 (w), 1532 (w), 1426 (m), 1383 (w), 1266 (m), 1230 (s), 1203 (m), 1180 (w), 1095 (m), 1060 (w), 845 (w), 698 (m), 579 (m), 549 (m), and 525 (vs) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃, ppm) δ 4.70 (t, *J* = 4.55 Hz, 2H), 3.95 (t, *J* = 4.55 Hz, 2H), 3.80 (m, 6H), 3.76 (t, *J* = 4.60 Hz, 2H), and 3.59 (s, 3H); ¹³C-NMR (500 MHz, CDCl₃, ppm) δ 164.12 (O=C-O-), 163.79 (O=C-OH), 145.57 (2C), 145.52 (2C), 145.33 (3C), 145.32 (3C), 145.22 (4C), 145.15 (3C), 144.84 (2C), 144.75 (C), 144.68 (4C), 144.57 (2C), 144.54 (2C), 143.90 (2C), 143.88 (2C), 143.04 (3C), 142.99 (4C), 142.96 (2C), 142.23 (3C), 142.01 (2C), 141.97 (2C), 140.93 (3C), 140.92 (3C), 139.36 (2C), 138.90 (2C), 72.19, 71.88 (fullerenyl sp³ carbon, 2C), 70.89, 70.64, 70.33, 69.70, 68.34, 65.99, 58.78, and 52.51.

3.3.15. Synthesis of Methoxyethyleneglycol-(20-oxo-4,7,10,13,16-pentapropyl-4,7,10,13,16,19-hexaaza-nonadecan-19-yl)[60]fullerenyl Malonate, $C_{60}(>>ME_1N_6C_3)$ (**17**) and $C_{60}(>>ME_1N_6^+C_3)$ (**1'**)

The compound of 2-[60]fullerenyl-3-(2-methoxyethoxy)-3-oxopropanoic acid **15** (0.15 g, 0.17 mmol) was taken into a round bottom flask containing anhydrous tetrahydrofuran (20 mL). To this reaction mixture was added thionyl chloride (0.03 g, 2.55 mmol) under N_2 atmosphere and refluxed for a period of 2.0 h. An excessive amount of thionyl chloride was removed on rotavap. Fresh anhydrous tetrahydrofuran (20 mL) was added. To this reaction solution was added slowly *N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amine **6** (0.08 g, 0.17 mmol) at 0 °C. It was warmed gradually to room temperature and stirred at this temperature for a period of 3.0 h. The resulting solution was concentrated on rotavap with the residue washed sequentially with hexane (10 mL), methanol (2×10 mL), and toluene (3×10 mL) to fully remove unreacted *N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amine and decarboxylated C_{60} byproducts. A relatively pure $C_{60}(>>ME_1N_6C_3)$ (**17**) was obtained in 47% yield (0.11 g). It was subsequently treated with CF_3COOH to result in pentacationic quaternary ammonium–trifluoroacetate salt, $C_{60}(>>ME_1N_6^+C_3)$ (**1'**), specifically, for NMR measurements. Spectroscopic data of the compound **17**: FT-IR (KBr) ν_{max} 3670 (br, s), 2977 (w), 2917 (w), 2878 (w), 2814 (w), 1792 (w), 1750 (s), 1708 (s), 1681 (m), 1541 (w), 1427 (m), 1390 (w), 1366 (m), 1267 (s), 1250 (s), 1231 (s), 1186 (m), 1114 (w), 1059 (w), 1020 (w), 864 (w), 743 (w), 701 (m), 572 (m), 552 (m), and 525 (vs, a characteristic band of C_{60} monoadduct) cm^{-1} . Spectroscopic data of the compound **1'**: 1H -NMR [500 MHz, $CDCl_3$ -toluene- d_8 -TFA (3:1:1), ppm] δ 4.22–4.68 (m, br, 2H), 3.90–4.05 (m, br, 2H), 3.22–3.75 (m, br, 35H), 1.28–1.36 (m, br, 12H), and 0.97 (m, br, 18H); ^{13}C -NMR [500 MHz, $CDCl_3$ -toluene- d_8 -TFA (3:1:1), ppm] δ 167.72 (O=C-O-), 166.46 (O=C-NH-), 148.00 (2C), 145.54 (2C), 145.30 (2C), 145.29 (2C), 145.24 (2C), 145.14 (2C), 145.02 (2C), 144.73 (4C), 144.68 (4C), 144.53 (2C), 143.98 (2C), 143.77 (2C), 143.27 (2C), 143.11 (C), 143.04 (2C), 143.01 (2C), 142.98 (C), 142.87 (2C), 142.44 (2C), 142.22 (2C), 142.12 (2C), 142.01 (2C), 141.17 (2C), 140.98 (2C), 140.51 (2C), 137.92 (4C), 136.50 (2C), 70.47, 70.23 (fullerenyl sp^3 carbon, 2C), 70.08, 69.73, 64.65, 64.40, 58.94, 58.68, 38.43, 31.84, 22.04, 21.40, 16.66, and 10.10 (quaternary aminocarbon peaks were low in intensity).

3.3.16. Synthesis of Pentacationic Methoxyethyleneglycol-(20-oxo-4,7,10,13,16-pentapropyl-4,7,10,13,16,19-hexaaza-nonadecan-19-yl)[60]fullerenyl Malonate Quaternary Methyl Ammonium Salt, $C_{60}(>>ME_1N_6^+C_3)$ (**1**)

A solution of [60]fullerenyl malonate quaternary ammonium–trifluoroacetate salt **1'** (100 mg) in chloroform (50 mL) was neutralized with aqueous potassium carbonate (10%, 50 mL). The resulting [60]fullerenyl malonate **17** was then dissolved in a mixture of anhydrous chloroform (30 mL) and dimethylformamide (15 mL). To this reaction, an excess amount of iodomethane was added in several portions over the reaction period and stirred at 45 °C for 3.0 days. At the end of the reaction, the solvent was removed on rotavap to yield pentacationic $C_{60}(>>ME_1N_6^+C_3)$ (**1**). Spectroscopic data: MALDI-TOF-MS (sinapic acid as the matrix, rel. intensity) m/z 673 (20%), 697 (50%), 721 ($C_{60}H^+$, 100%), 734 (80%), 746 (15%), 761 (10%), 772 (10%), 874 [$C_{60}(>>H(C=O)NHCH_2CH_2N^+$ -propylMe $_2$), 20%], 1442 [$(C_{60}H)_2^+$ cluster], 1565, 1634, 1709, 1769, 1851, 1930 (M^+-I^-); FT-IR (KBr) ν_{max} 3688

(br, s), 2918 (s), 2870 (m), 2840 (m), 2807 (w), 1784 (w), 1736 (vs), 1663 (s), 1574 (w), 1433 (m), 1383 (w), 1252 (w), 1187 (m), 1163 (s), 1126 (m), 1090 (m), 1031 (s), 842 (w), 762 (w), 704 (w), 661 (w), 569 (w), and 524 (vs, a characteristic band of C₆₀ monoadduct) cm⁻¹; UV-Vis (DMF, cutoff at 268 nm, 2.0 × 10⁻⁵ M) λ_{max} 323 nm (shoulder peak); ¹H-NMR [500 MHz, DMSO-*d*₆-toluene-*d*₈ (2:1), ppm] δ 3.80–4.25 (m, br, 16H), 2.90–3.80 (m, br, 39H), 1.50–1.80 (m, br, 12H), and 0.88–0.99 (m, br, 18H). We found that electronic interferences of iodide anions in a high quantity with the fullerene cage or possible partial electron-transfer events prohibited the detection of fullereryl carbon peaks (in low signal intensity).

3.3.17. Synthesis of Pentacationic Methoxy-tri(ethyleneglycol)-(20-oxo-4,7,10,13,16-pentapropyl-4,7,10,13,16,19-hexaaza-nonadecan-19-yl)[60]fullereryl Malonate Methyl Quaternary Ammonium Salt, C₆₀(>ME₃N₆⁺C₃) (2)

Synthesis of the compound **2** was carried out by using a similar procedure as that of **1** except methoxytriethyleneglycol ester was applied instead. Spectroscopic data: MALDI-TOF-MS (sinapic acid as the matrix, rel. intensity) *m/z* 698 (10%), 721 (C₆₀H⁺, 100%), 735 (C₆₀>H₂, 20%), 749, 773, 782, 789, 809, 874 [C₆₀(>H(C=O)NHCH₂CH₂N⁺-propylMe₂)], 914, 940, 995, 1027, 1054, 1278 (w), 1395 (w), 1417 (vw), 1499 (vw), 1792 (vw), and 2019 (vw, MH⁺-I⁻); FT-IR (KBr) ν_{max} 3424 (vs), 2967 (m), 2925 (m), 2874 (m), 2824 (w), 1738 (s), 1681 (s), 1628 (s), 1454 (vs), 1429 (s), 1383 (m), 1064 (s), 1028 (s), 941 (m), 727 (m), 572 (m), and 524 (vs, a characteristic band of C₆₀ monoadduct) cm⁻¹; UV-vis (DMF, cutoff at 268 nm, 2.0 × 10⁻⁵ M) λ_{max} 323 nm (shoulder peak); ¹H-NMR [500 MHz, DMSO-*d*₆-toluene-*d*₈ (2:1), ppm] δ 3.80–4.20 (m, br, 16H), 2.90–3.80 (m, br, 47H), 1.50–1.75 (m, br, 12H), and 0.88–0.99 (m, br, 18H). We found that electronic interferences of iodide anions in a high quantity with the fullerene cage or possible partial electron-transfer events prohibited the detection of fullereryl carbon peaks (in low signal intensity).

4. Conclusions

We have designed and synthesized two analogous pentacationic [60]fullereryl monoadducts, C₆₀(>ME₁N₆⁺C₃) (**1**) and C₆₀(>ME₃N₆⁺C₃) (**2**), with variation of the methoxyethyleneglycol length. Each of these derivatives bears a well-defined number of cationic charges aimed to enhance and control their ability to target pathogenic Gram-positive and Gram-negative bacterial cells. The intrinsic nature of the high charge number and increased water-solubility of the precursor arm intermediates hindered the efficiency of their reactions with a highly hydrophobic C₆₀ cage having low compatibility in polar solvents. Furthermore, consecutive ethylamino group linkage in a structure of *N,N',N,N,N,N*-hexapropyl-hexa(aminoethyl)amine (C₃N₆) largely increased its electron-donating capability that gave complication in forming an insoluble partial charge-transfer complex with C₆₀ during the reaction and workup procedures. After many attempts, we found a circumventive solution by the use of partially quaternized C₃N₆ prior to the reaction with C₆₀ coupled with the modification of C₃N₆ arm using propyl groups, instead of methyl or ethyl groups, to provide a well-balanced hydrophobicity–hydrophilicity character of pentacationic precursor intermediates and better compatibility with the C₆₀ cage moiety.

Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/17/5/5225/s1>.

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Sample Availability: Samples of the compounds **1** and **2** may be available from the authors.

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