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Functionalized Fullerenes in Water. The First 10 Years of Their Chemistry, Biology, and Nanoscience

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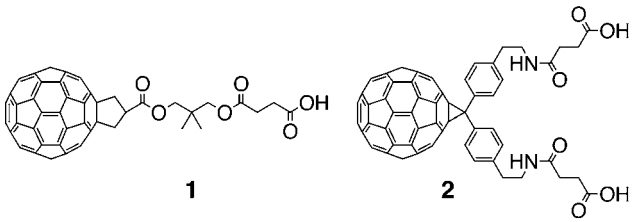
ABSTRACT

Fullerenes are entirely insoluble in water, but suitable functionalization makes the molecules soluble. Studies on water-soluble fullerene derivatives led to the discovery of the interaction of organofullerenes with DNA, proteins, and living cells, which was first reported in the summer of 1993. Subsequent studies have revealed interesting biological activity aspects of organofullerenes owing to their photochemistry, radical quenching, and hydrophobicity to form one- to three-dimensional supramolecular complexes. In these areas of research, synthetic organic chemistry has played an important role in the creation of tailor-made molecules.

Introduction

Ten years have elapsed since the first reports on the biological activity of water-soluble organo-functionalized [60]fullerenes (C_{60} hereafter) in the summer of 1993. Photodriven DNA cleavage,[1] enzyme inhibition,[1−3] and cytotoxicity[1] were reported for compounds **1** and **2** (Figure 1). Since then, numerous papers have reported on their interaction with biological targets.[4] These studies have aroused wide interest in the behavior of fullerenes in water and created a new area of science of functionalized fullerenes that is rapidly expanding toward clinical and materials applications. Several fundamental properties of fullerenes form the background of such interest in fullerenes in water: extremely high hydrophobicity, high cohesive force between fullerene molecules,[5] photoactivity,[6] ability to accept and release electrons,[7] and relatively high reactivity that allows structural modifications.[8] This Account summarizes the current status of the science of water-soluble fullerenes that started a decade ago as curiosity-driven studies and is now flourishing as an area of transdisciplinary research.



1 **2**

FIGURE 1. Mono- and dicarboxylic acids **1** and **2** that were used for the first biological studies of water-soluble fullerenes.

The Initial Discovery

Long after the initial proposal by Osawa in 1970,[9] and the mass spectroscopic detection by Curl, Kroto, and Smalley in 1985,[10] the synthesis of a macroscopic quantity of fullerenes by Krätschmer and Huffman in 1990 suddenly fueled the interest of experimentalists in fullerenes.[11] One of the future applications that came immediately to mind was their potential use in biology. An obvious obstacle

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Hiroyuki Isobe was born in Tokyo, Japan, in 1970. He received his B.S. and M.S. degrees from Tokyo Institute of Technology and received his Ph.D. degree from The University of Tokyo. During that time he joined Professor Daniel Kahne's group at Princeton University as a summer student (1996). In 1998, he became an assistant professor of The University of Tokyo. He received the first IUPAC Prize for Young Chemists in 2000 and the first Young Scientist's Research Award in Natural Product Chemistry in 2001. His current research interests include the synthesis of nanometer-sized organic compounds and development of their functions in bioorganic and materials chemistry.

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Table 1. Solubility of C_{60} in Various Solvents[12]

solvent	solubility (mol/L)
1-chloronaphthalene	7×10^{-2}
1,2-dichlorobenzene	4×10^{-2}
carbon disulfide	1×10^{-2}
chlorobenzene	1×10^{-2}
toluene	4×10^{-3}
benzene	2×10^{-3}
decane	1×10^{-3}
acetone	1×10^{-6}
water	2×10^{-24}

was the complete lack of solubility of fullerenes in water. As shown in Table 1, C_{60} is insoluble both in water and in polar solvents.[12] Two approaches were immediately sought to increase the solubility: one utilizes a solubilizing agent such as poly(*N*-vinylpyrrolidone)[13] or cyclodextrins,[14] and the other relies on introduction of hydrophilic function-(s) by chemical modification of the molecule. In the latter approach, the attachment of amino or hydroxyl groups was immediately found to be an easy and straightforward method.[15] The reactions, however, afforded an inseparable mixture comprising many isomers as well as compounds possessing different numbers of heteroatomic groups. Carbon−carbon bond-forming modifications of fullerenes were subsequently found to be more selective, producing well-defined products.[16] Compounds **1** and **2** represent the compounds in this category synthesized at the very first stage of the development.

Because of our interest in biological applications of strained molecules in the late 1980s,[17] we were intrigued by the interaction of fullerenes with biological targets by way of the strained double bonds or their photoactivity. We synthesized compound **1** by a [3 + 2] cycloaddition[16] and, together with Sugiura of Kyoto University and a group of people at Mitsubishi Chemical Co., tested it for activity with DNA, enzymes, and mammalian cells. We thus discovered that the compound shows significant biological activity:[1] Compound **1** (and later its C_{70} analogue)[18] suppresses cell growth and inhibits activity of various enzymes (including HIV protease, as we found later).[19] We then noted considerable effects of incident light during experimentation. Among various biological targets, DNA showed the sharpest response to visible light irradiation: brief light exposure of a mixture of **1** and a plasmid DNA resulted in single-strand nicking, and longer exposure led to double-strand cleavage, largely at guanine sites, which is the nucleobase most susceptible to oxidation (Figure 2). No cleavage was observed in the dark. Later studies indicated that the cleavage may be caused both by electron transfer from nucleobases and by active oxygen species generated by fullerene-sensitized activation of molecular oxygen in the medium.[20,21] We later found that a simple carboxylic acid such as **1** does not bind to a plasmid DNA, and hence it was unlikely that the DNA was undergoing direct chemical reaction with **1**.[22] In contrast to our initial naive assumption, the strain energy in fullerene molecules has so far not been shown to be important in any biological applications.

Simultaneously with our report, two papers from researchers in San Francisco[2] and Atlanta,[3] using the Wudl

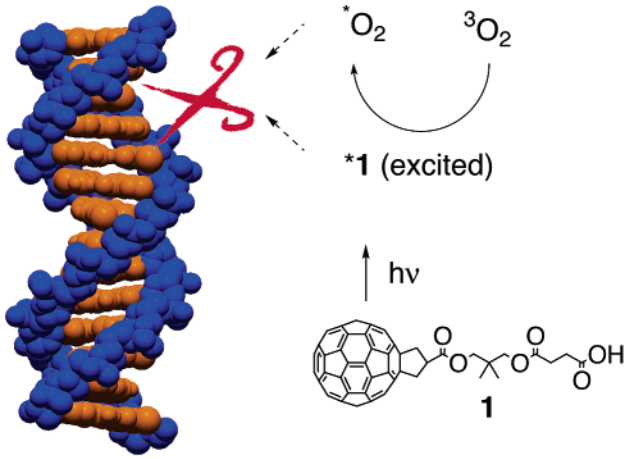


FIGURE 2. Oxidative cleavage of DNA with fullerene **1**.

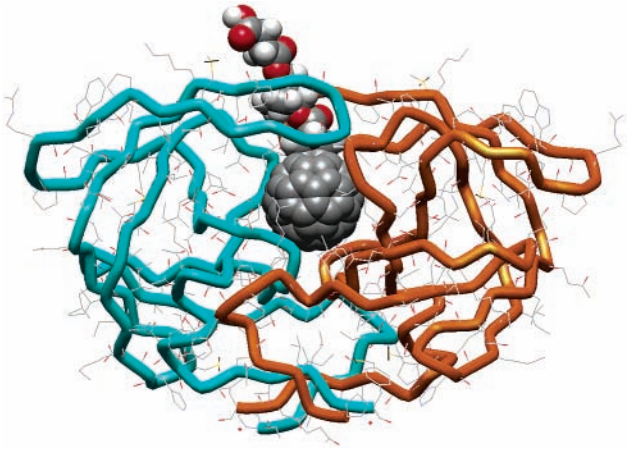


FIGURE 3. 3D model of the complex between **1** (space-filling model) and HIV protease (tube and stick models).

compound **2**, reported inhibition of HIV protease. The dicarboxylic acid **2** acts as a reversible inhibitor of the enzyme through binding to the cleft-like active site of the dimeric enzyme in competition with the natural peptide substrate. This shape-recognition binding is illustrated in Figure 3 for our monocarboxylic acid **1**, which is an equally potent inhibitor of the enzyme.[19] A dendritic variation of the fullerene inhibitor is being developed for treatment of drug-resistant HIV.[23]

Pharmacokinetics and Toxicity of Water-Soluble Fullerenes. From the beginning of this research, there has been a concern about the adverse effects of water-soluble fullerenes, which has gone together with an optimistic view on future development. Toxicity was the primary concern for people working with fullerenes at the bench. Though earlier studies on C_{60} itself suggested low toxicity,[24] it was unclear if water-soluble fullerenes are also innocuous molecules. Obviously, pharmacokinetic studies of water-soluble fullerenes were first necessary.

To this end, we synthesized in 1994 the first radiolabeled water-soluble fullerene, **1-C14**,[25] by taking advantage of the dipolar trimethylenemethane chemistry.[26] Intravenous injection of **1-C14** to mice provided the first pharmacokinetic information on water-soluble fullerene (Fig-

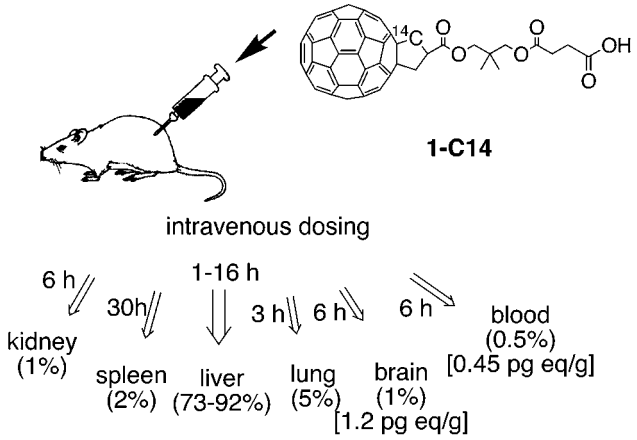
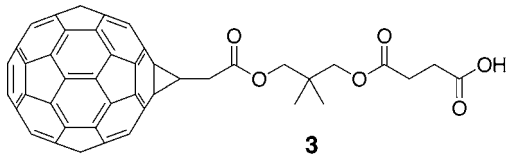


FIGURE 4. Pharmacokinetics of ^{14}C-labeled fullerene carboxylic acid **1-C14**.

ure 4). The compound quickly migrated through the body and accumulated mainly in the liver after a few hours. Importantly, the study suggested for the first time that the fullerene molecule can pass through the blood−brain barrier, and that was later confirmed by others.[27,28] This behavior stands in sharp contrast to that of the parent fullerene, which was absorbed by serum protein in blood quickly after injection.[24b,29] The pharmacokinetic studies have shown that organofullerenes are excreted either slowly or rapidly, depending on the substituents, which may be suitably modified under the capacity of medicinal chemists.[30]



With these data in hand, we examined the toxicity of the carboxylic acid **3** to find that the molecule does not show serious acute toxicity. Thus, upon injection of up to a 500 mg/kg quantity of **3** into female mice, all animals survived for 1 week.[25] Later studies by others[31] also indicated that the fullerene molecules do not show appreciable short-term toxicity. Long-term toxicity has so far not been reported. Though fullerenes were initially considered to be a man-made, unnatural substance, it is now known that we have already been exposed for a long time to these compounds that are located in the terrestrial or extraterrestrial space.[32]

Further Developments in Biological Studies

Selective Binding to DNA. The initial discoveries made for the two simple molecules **1** and **2** quickly led to studies on further applications using more complex molecules. In 1994, in collaboration with Hélène in Paris, we connected a fullerene molecule to a 14-mer DNA sequence that is complementary to a double helix. The fullerene−DNA conjugate **4** was found to bind to the target DNA (Figure 5),[33] cleaving the strand at guanine sites in the position expected from the triple helix formation. A similar

FIGURE 5. Triple-helix-forming fullerene−DNA conjugate **4** (coded with purple color), its 3D binding model with target DNA (coded with blue, green, and orange), and site-selective photocleavage of DNA (as indicated by arrows and coded with orange).

result was reported later by Sigman et al.[34] We initially expected that the fullerene moiety might stabilize the triple helix, but recent studies suggested that this is not necessarily the case.[35] Fullerene molecules bearing an intercalating group have been shown to cleave DNA more efficiently than the parent C_{60}.[36]

Our finding on the photocytotoxicity of compound **1** paved the way to the use of fullerene in photodynamic cancer therapy. Research by Ikada and Tabata in Kyoto demonstrated a considerable effect of C_{60} bearing polyether side chains in shrinking skin cancer in mice.[30] Chiang in Taipei also pursued this line of research toward clinical applications.[37] Inhibition of virus growth has also been reported.[38] The actual biological targets of these approaches still remain unclear, though DNA may be a possible candidate.

Gene Delivery. During the foregoing studies, we noticed that attachment of fullerene to DNA causes aggregate formation in a buffer solution. With this information, we speculated that suitably functionalized fullerene might be useful for delivery of DNA into living cells,[39] and hence for gene therapy of diseases. This idea was put into action in collaboration with Okayama in our medical school. Lipids bearing a cationic amine side chain capable of binding to DNA through electrostatic interaction are the generic structures of common chemical DNA vectors. On the basis of the unique properties of fullerenes, we conjectured that charged fullerenes might offer a new possibility in this exciting area of biomedical research.

We synthesized a series of fullerenes that bear amino side chain(s) and examined their DNA binding ability and their transfection capability.[22,40] Some representative examples are shown in Figure 6: The C_2 compound **5** was designed to be a large C_2-chiral molecule that may recognize the chirality of large molecules and molecular assemblies such as double-helical DNA (this property of the molecule is yet to be demonstrated). Compound **6** is its more densely functionalized optically active congener. The ammonium ion **7** represents a minimal structure for nitrogen-containing water-soluble fullerenes.[41] Compound **8** possesses as many as eight nitrogen atoms and is available in one step from C_{60} in quantitative yield (vide infra).[42]

When a solution of a 4.5-kbp plasmid DNA with a reporter gene was mixed with a solution of the two-

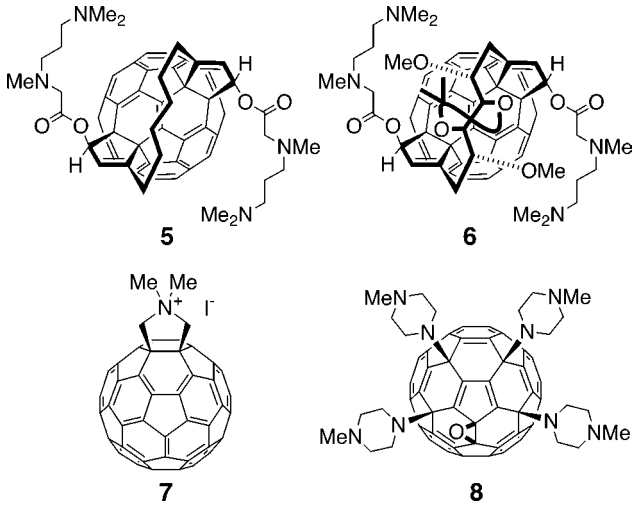


FIGURE 6. Representative DNA-binding fullerenes examined for transfection.

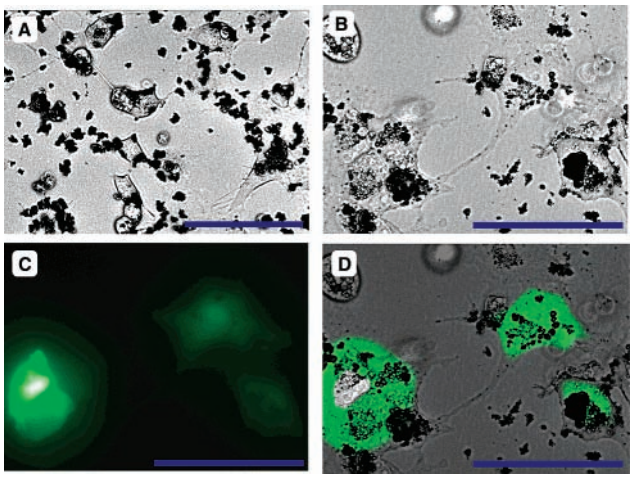
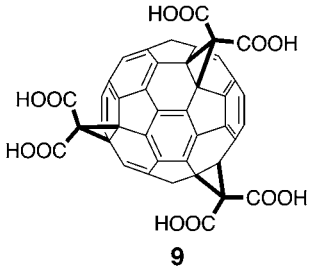


FIGURE 7. Delivery of GFP plasmid into COS-1 cells with **5**. Blue bars represent 100 μm. (A) Differential interference contrast (DIC) micrograph of COS-1 cells and small black particles (1−10 μm) of the fullerene−DNA complex after 1 h of transfection time. (B) DIC micrograph of COS-1 cells after 2 d incubation time. (C) Fluorescence image of the same area as in B. (D) Superimposed photograph of B and C. Round black clumps of 0.5−3 μm size observed in the fluorescent cells contain fullerene materials. Adapted from ref 22.

handed tetraminofullerenes **5** and **6** in buffer solutions, particles of 1−10 μm diameter formed, as observed by optical microscopy (Figure 7). As this mixture was incubated with dividing mammalian cells, the dark-colored fullerene−DNA particles were taken into the cells, and protein production ensued. Screening of various cell lines indicated that the efficiency of this first-generation fullerene vector is comparable to or better than that of the commercially available lipid-based vectors (e.g., lipofectin).

Tour at Rice University treated double-stranded DNA with aminofullerene **7** to find that the fullerene molecules stick to DNA without much change in the linear structure of the DNA, as studied by transmission electron microscope.[41] We found that **7** and **8** bind strongly to DNA but are not effective for gene delivery.[40]

Neuron Protection via Radical Scavenge. An intriguing recent discovery is the ability of the fullerene hexacarboxylic acid **9** to act as a drug for neurodegenerative diseases such as Alzheimer's, Parkinson's, or amyotrophic lateral sclerosis (ALS).[27] The activity is considered to arise from trapping of hydroxy radicals by the fullerene molecule.[37] Fullerene drugs for ALS treatment are currently being investigated as a drug candidate by a Canadian company.



Endohedral Metal Complexes for Biology. Metal-containing fullerenes are attracting attention for possible clinical applications. Wilson in Houston has demonstrated the use of endohedral metallofullerene as an in vivo radiotracer. Biodistribution studies of $^{166}Ho_x@C_{82}(OH)_y$ showed uptake of the polyhydroxylated fullerene by bone without clearance.[28] The use of metallofullerenes as a magnetic resonance imaging (MRI) contrast agent is another area of extensive current studies. Shinohara in Nagoya[43] found that $Gd@C_{82}(OH)_n$ is much more efficient than the commercial contrast agents (e.g., Magnevist, gadolinium diethylenetriaminepentaacetic acid). Because of the very large surface in contact with water, the metal-containing fullerene accelerates spin relaxation (T_1) of water protons that surround the fullerene molecule. Bolskar and Wilson reported polycarboxylic acid derivatives of $Gd@C_{60}$ to also serve as an excellent contrast agent.[44] The biggest obstacle toward practical applications is the entire lack of synthetic methods for large-scale production of endohedral metal complexes.

Molecular Assembly of Fullerenes in Water

Are the "water-soluble" fullerenes truly "soluble" in water? This question has lingered for a long time in the mind of scientists working in the field. Studies so far have revealed various intriguing supramolecular structures of fullerene aggregates, some of which were unknown for conventional surfactants and lipid molecules. Various new discoveries have been made in recent years with respect to the formation of one- to three-dimensional supramolecular objects, including vesicles, rods, globules, membranes, and linear assemblies.

Langmuir—Blodgett Film Formation. The formation of molecular layers of fullerenes and their derivatives has attracted the attention of scientists ever since the beginning of materials research on fullerenes, yet earlier attempts to make uniform monolayers of C_{60} were rather unsatisfactory.[45] Together with Matsumoto at Tsukuba, we found that our amphiphilic fullerene carboxylic acid **1** forms a uniform Langmuir−Blodgett film at the air−water interface.[46] Fullerene carboxylic acid **1** can be deposited

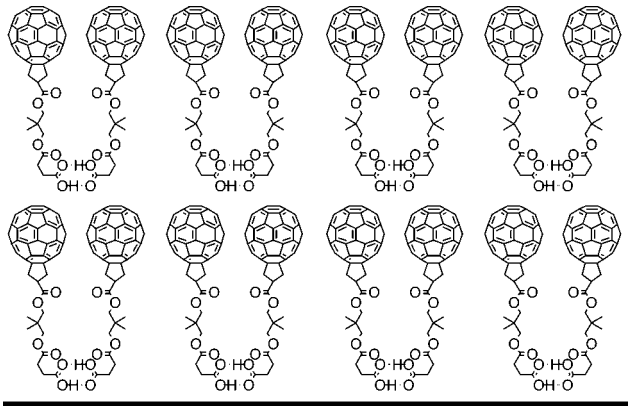


FIGURE 8. Langmuir−Blodgett film formation (Z-type) of fullerene carboxylic acid **1**.

on mica to form Z-type LB films (Figure 8). Atomic force microscopic (AFM) analysis of the monolayer indicated that the surface contains far less defects than the LB film made from unmodified C_{60} under similar conditions.

Nanorods and Nanoparticles. Tour dispersed a DMSO solution of a cationic amphiphile **7** in benzene to discover the formation of a nanorod structure.[41] The nanorods have a diameter of 14−120 nm and a length of over 70 μm. When the dispersion was sonicated, vesicles of various sizes and shapes formed. The thickness of the vesicle wall (3−6 nm) suggested a multilamellar structure. In a similar line of research, Shinkai synthesized a long aliphatic ammonium bearing a C_{60} group on its hydrophobic terminal, and noted the formation of vesicles of diameter ranging from 20 to 50 nm.[47] The properties of the vesicle appear to depend largely on the quaternary ammonium moiety of the molecule. Prato et al. recently reported that cationic amphiphiles form nanospheres and nanorods.[48]

Bilayer Vesicle Formation. $C_{60}Ph_5H$ (**10**) is an interesting hydrocarbon in that its anion, $C_{60}Ph_5K$ (**11**), dissolves freely in water (Scheme 1). The fact that the cyclopentadienyl anion remains deprotonated in water was already surprising, since the ordinary cyclopentadienyl anion is immediately protonated upon addition to water. Much more interesting was that it dissolves in water not as a unimer but as spherical aggregates, as we observed by AFM (Figure 9).[49] This unexpected finding, made by Sawamura in our group, suggested that fullerene may create entirely unconventional vesicles in water.

Careful AFM and laser light scattering (LLS) studies of the solution of **11** indicated that the solution contains bilayer vesicles and that their size distribution is quite narrow.[50] Thus, upon slow injection of a THF solution to water, $Ph_5C_{60}K$ forms spherical bilayer aggregates of 34-nm average diameter, composed of about 13 000 fullerene molecules (Figure 9), as determined by LLS in Chu's laboratory in Stony Brook. This ability of $Ph_5C_{60}K$ to form vesicles in water is most intriguing, since the molecule lacks both the head−tail structure and long alkyl chain(s) characteristic of conventional amphiphilic surfactants and lipids. The Ph_5C_{60} anion amphiphile is noteworthy for its carbon framework, high structural rigidity, spherical hydrophobic moiety, and lack of heteroatom hydrophilic

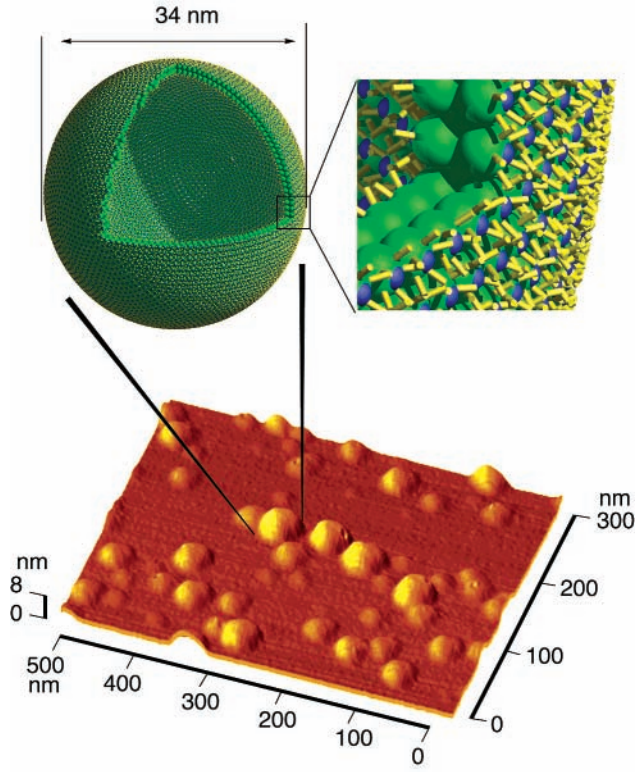
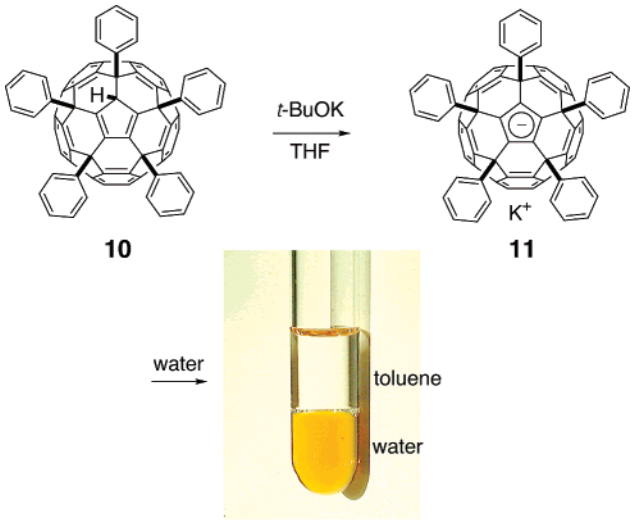


FIGURE 9. AFM image of $Ph_5C_{60}K$ vesicle and bilayer model based on LLS results. For color coding of the molecules, see Figure 10. Adapted from ref 50.

Scheme 1. Yellow-Colored 11 Dissolved in Water



The yellow-colored **11** dissolves in water.

sites. In addition, the fullerene moiety is highly hydrophobic but not lipophilic (note that fullerene does not dissolve in hexane, cf. Table 1).

Figure 10 illustrates another difference: The conventional lipid (a) consists of two parts, hydrophobic side chains composed of methylene groups and a hydrophilic site composed of heteroatom anion(s). On the other hand, the new amphiphile (b) possesses, in addition to these two sites, five organic groups that protrude toward the outside of the vesicle, which provide a new opportunity

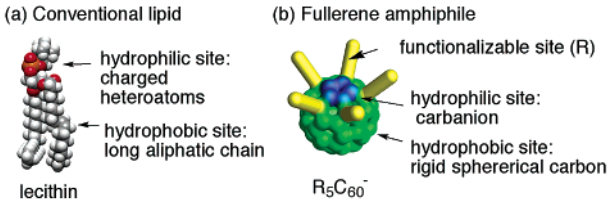


FIGURE 10. Molecular models of (a) a representative biological lipid (lecithin; C, gray; H, white; O, red; N, blue; P, orange) and (b) a fullerene amphiphile $R_5C_{60}^-$. Neutral carbons are shown in green, charged cyclopentadienide in blue, and R substituents in yellow.

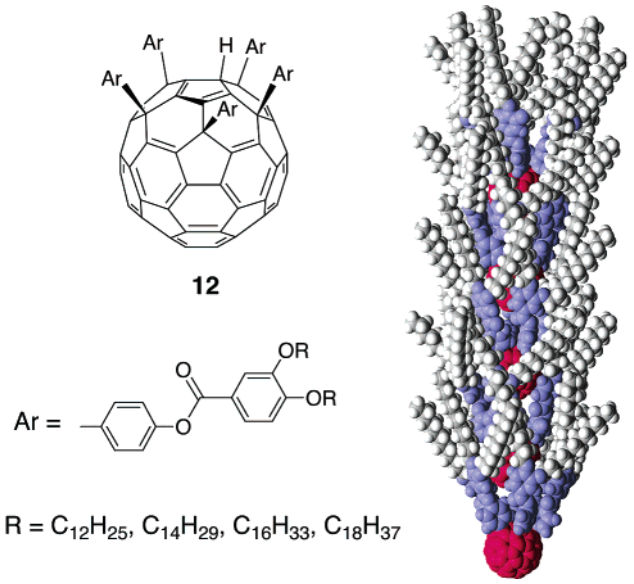


FIGURE 11. Nanoshuttlecock molecule **12** and a 3D model of a stack of five molecules.

to obtain functional vesicles through functionalization of the amphiphile.

The phenyl groups in **10** can be modified into larger groups to form a cavity that can recognize another C_{60} molecule. This molecule **12** looks like a badminton shuttlecock and stacks with itself to form a one-dimensional array of fullerene molecules both in crystals and in liquid crystals (Figure 11), as studied in collaboration with Kato in our engineering school.[51] We expect that, with suitable modifications, such one-dimensional stacking would also be useful for the formation of supramolecular structures in aqueous solutions.

DNA/Fullerene Nanoparticles. The aforementioned experiments of gene delivery demonstrate the ability of the fullerene **5** to condense and to release DNA, and the molecular nature of the DNA condensation/release process was probed by test tube experiments and AFM, as performed in collaboration with Iwasawa in our department.[52] When we add 0.5 equiv/base pair of the fullerene **5**, a single-plasmid DNA (Figure 12a) is condensed into a globular object that can be seen in Figure 12b as a flat disk of 3 nm thickness and 50 nm diameter. Upon further addition of **5**, many single-molecule DNA condensates gather together to form micrometer-sized objects (Figure 12c). The DNA molecules are released from these particles (Figure 12d) upon extractive removal of the fullerene by $CHCl_3$. The results provide a chemical model of the DNA

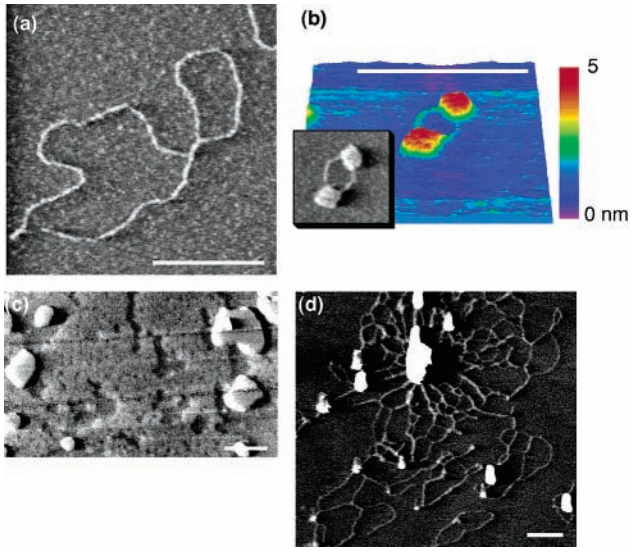
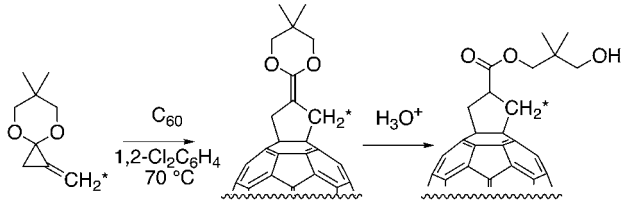
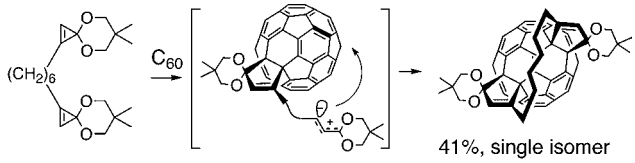


FIGURE 12. Condensation of 4-kbp plasmid DNA (a) into globules with increasing amount of **5** (b,c) and its release upon removal of fullerene derivative (d). White bars in the images represent 200 nm. Adapted from ref 52.

Scheme 2



Scheme 3

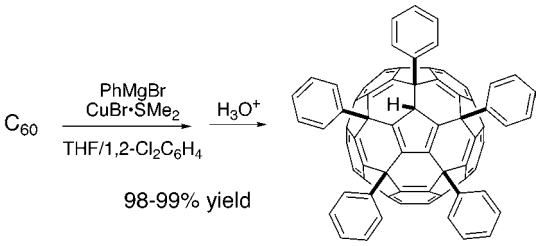


condensation and release processes in the biological experiments.

Estimation of the volume of each fullerene−DNA condensate indicates that the fullerene folds the DNA with very little increase of volume of DNA, as compared with the natural histone protein that forms a chromatin structure with a 10−100 times increase of the volume of DNA. The formation of globular condensates composed of a single to a few DNA molecules is unique among other DNA-condensation studies based on lipid and dendrimeric molecules, which create much larger and less structurally defined DNA condensates.[53]

Studies on a series of DNA-binding aminofullerenes recently showed that DNA condensation is required but is not sufficient for successful transfection.[40] We are speculating presently that, in the cytoplasm, the ester linkage in the fullerene **5** gradually yields to hydrolase activity, detaching the tetramino DNA-binding sites from **5**, and hence results in the release of DNA from the aggregate.

Scheme 4



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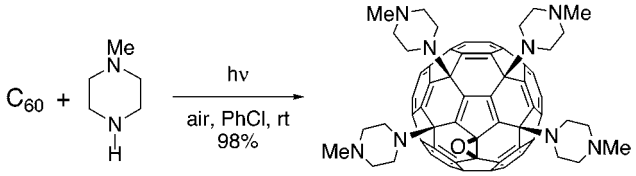
Scheme 5



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Fullerene-Binding Antibody. While HIV protease is a naturally occurring receptor of fullerene, a fullerene binding protein has been elicited by Erlanger's group of Columbia University through immunization.[54] They immunized mice with a C_{60}−bovine thyroglobulin (>600 kDa) conjugate to obtain the desired IgG isotype antibody. The crystal structure of the Fab fragment of the anti-fullerene antibody revealed that a shape-complementary clustering of hydrophobic amino acids forms a fullerene binding site.[55] A modeling study shows participation of the induced fit mechanism in the binding process. Interestingly, a subpopulation of the antibodies also recognizes C_{70}. Such antibodies also bind to carbon nanotubes.[56]

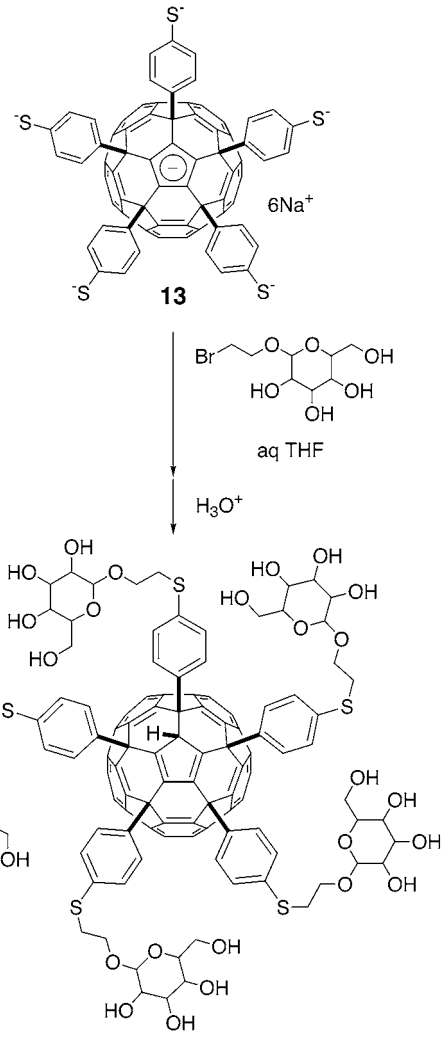
Fundamental Synthetic Methodology

With our background in synthetic organic chemistry,[57] we have always tried to develop a new synthetic methodology whenever we design new functions of fullerene derivatives. Our entry to the field was made possible by the use of the dipolar trimethylenemethane chemistry, with which we synthesized compounds **1** and **1-C14** starting from an alkoxy-substituted methylenecyclopropane (Scheme 2).[26]

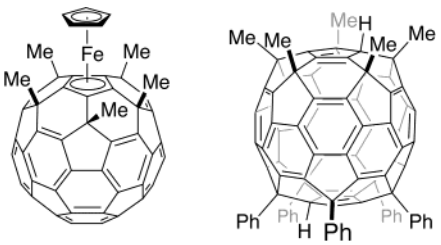
The C_2-chiral two-handed fullerene **5** that we used for the gene delivery studies was synthesized by a regioselective double [3 + 2] cycloaddition of a bis-cyclopropenone acetal (via vinyl carbene intermediates) (Scheme 3).[58,59]

In 1996, we reported a remarkably effective penta-addition reaction of a phenylcopper reagent with C_{60} that takes place to give **10**.[60,61] The reaction is so clean that an analytically pure product was isolated in 98−99% yield on a 10-g scale after simple washing of the crude product with water and hexane (Scheme 4). Among numerous methods for chemical modification of fullerenes and other carbon clusters, there are only two reactions that take place in quantitative yield on the basis of the amount of the carbon cluster used (not the consumed material): the penta-addition reaction (tris-addition product from C_{70})[62]

Scheme 6



and the oxygenative photo-tetra-addition of a secondary amine (Scheme 5).[42,63] The former reaction is particularly general as to the R group (methyl, alkenyl and aryl groups). By the use of hexaanion **13** of a pentathiol, one can achieve coupling of the intrinsically hydrophobic fullerene molecule and the water-soluble unprotected sugar moiety in aqueous solution.[64] The new method for the synthesis of a polysaccharide **14** will provide a new tool for the studies of polysaccharide displays. The reaction allowed us to synthesize the first ferrocene/fullerene hybrid **15**[65] and the first hoop-shaped aromatic structure **16**.[66]



15 **16**

Summary and Outlook

The science of water-soluble fullerenes has seen quick development in the past 10 years to a level that could not have been reached without the development of powerful methodologies for chemical modifications of fullerenes. The primitive molecular engineering in compounds **1** and **2** has seen dramatic improvement in recent years, culminating in the synthesis of much more complex compounds in high yield and on a large scale. Consequently, practical applications to clinical and materials uses are being seriously considered now. The science of water-soluble fullerene stimulated other studies for dissolving carbon nanotubes into water and organic solvents.

Rapidly growing knowledge on the chemical modification, biological significance, and materials applications notwithstanding, the largest barrier that has kept people from entering the field of fullerene research was the price of C_{60}. This problem is now resolved, because tons of C_{60} are produced by a Japanese venture company by burning toluene to produce fullerenes.[67] They have built a plant capable of producing 40 t/yr and plan production of 1500 t/yr in 2007.[68] With this huge supply of raw materials, the first mass application appeared earlier this year in the market in a very unexpected form: bowling balls with fullerene coating.[69] It is time for synthetic chemists to seriously ponder the development of truly efficient methods to functionalize fullerene derivatives on a large scale. The modern standard of "regio- and stereoselective synthesis" is yet to be achieved in fullerene chemistry.

In conclusion, research on water-soluble fullerenes is the field where the angstrom-sized world of bond-forming reactions is directly connected to the millimeter-sized world of materials and biology. The nanometer size of the fullerene molecules is large enough to allow implementation of a variety of "programs" into the molecules, and these programs guide the molecules to display the programmed functions as they interact with each other, with other molecules, or with living organisms. The field therefore waits for participation of scientists possessing high capability in synthesis, molecular design, materials studies, and biomedical applications. With active participation of scientists and engineers from various disciplines, the science and technology of fullerenes in water will see many more unexpected scientific discoveries and new commercial applications that will serve to increase the quality of life.

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