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Review Article

Biological Applications of Fullerenes

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Contents

1. Introduction

Over the past few years, many research groups have begun to investigate biological uses of fullerenes.[1] To understand these applications, it is useful first to review the general physical properties of this unique new class of molecule. Therefore, the first section of this survey provides a description of the general properties of fullerenes. Specific biological applications of fullerenes are then discussed in the following order: enzyme inhibition, antiviral activity, DNA cleavage, photodynamic therapy, electron transfer and miscellaneous uses. A section on fullerene metabolism, excretion, and toxicity is also included.

2. Properties of Fullerenes

2.1 Solubility

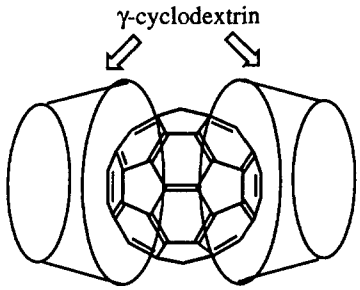
Several reports have appeared concerning fullerene solubilities in various solvents.[2–6] The solubility of C_{60} in polar solvents is known to be quite low.[2,6] On the other hand, a study of ^{14}C-labeled C_{60} reported that it was possible to form a suspension of C_{60} in water that was stable for long periods and could be delivered to cells.[7,8] Solubilization of C_{60} using cyclodextrins (up to a concentration of 80 μM) has also been reported.[9,10] Due to the size of C_{60}, the most common cyclodextrin, β-cyclodextrin, could not be used. Therefore, the larger internal radii of γ-cyclodextrins was employed to form a 2:1 complex (**1**) with C_{60}.[9] Alternatively, attachment of a biphenyl 'handle' to C_{60} allows the complexation and solubilization of fullerene derivative **2** with β-cyclodextrin.[11]

Other solubilization methods include the formation of inclusion complexes with calixerenes[12] and solubilization using the detergent Tween-20,[13] phospholipids,[14–16] micelles,[17,18] liposomes,[17] vesicles,[18] and polyvinylpyrrolidone.[19,20]

Several water soluble fullerene derivatives have been reported.[21–29] For example, polyamino and polyhydroxy fullerenes **3** and **4** have been studied,[28,29] as well as several monofunctional derivatives of C_{60} containing polar side chains. As a general rule, the greater the number of water solubilizing groups added to the fullerene, the greater the water solubility.

Most reports of 'water-soluble' fullerene compounds in biological studies do not address two important issues for drug development of any compound: (1) partition

Key words: fullerenes, enzyme inhibition, electron transfer, photodynamic therapy (PDT), metabolism.



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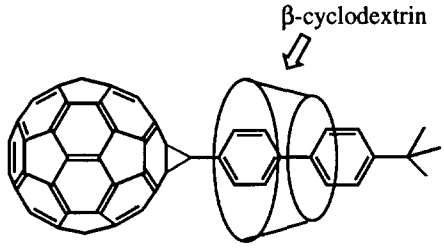

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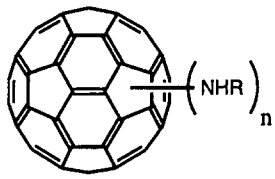

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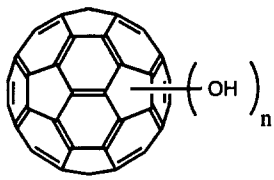

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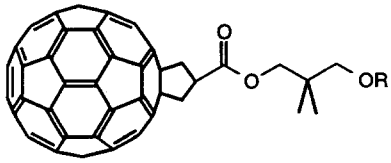


5a R = $CO(CH_2)_2CO_2H$
5b R = H
5c R = $CO(CH_2)_2CO_2H \bullet NEt_3$

coefficient ($\log P$) in octanol/water, a measure of relative lipophilicity, and (2) prospects for oral absorption. In spite of its reported water solubility, compound **5a** was only slightly absorbable orally in rats. Compound **5a** was also found to deposit in body tissues when injected intravenously due to its high lipophilicity.[30]

Bioactive fullerene compounds should be constructed so as to maximize the chances of low toxicity and advantageous adsorption, distribution, metabolism and elimination properties. Many of these parameters, such as toxicity and metabolism, are seldom more than educated guesses. However, it is generally accepted that there are certain desirable ranges of lipophilicity ($\log P$ values of -1 to $+2$), and water solubilities (solubilities $>$mg/mL) that are most likely to provide the best chances of oral absorption.[31,32] With the exception of studies on compound **5a**,[30] almost nothing has been published about the lipophilicity and water solubility characteristics that the fullerene moiety confers on analogues.

Partition coeffients ($\log P$)[33] in buffer/octanol and buffer/hexane should be measured by standard methods using UV absorption, to establish solubilities in the lipid and aqueous phases. These measured Log P values can then be added to commercially available CLOGP databases (such as BioByte Corp., Claremont, CA)[33] for use in prediction of the lipophilicity of future analogues. $\Delta \log P$ values[34,35] may then be calculated from the $\log P$ values determined in these two solvent systems, which have been proposed as improved measures of the ability of compounds to cross either the gut wall[36] or the blood–brain barrier.[35] It remains to be shown that good oral absorption of substituted fullerenes is possible. Fullerene derivatives whose measured lipophilicity/water solubility falls in advantageous ranges for oral bioavailability can then be used to test the hypothesis that fullerene-based drugs are possible.

2.2 Redox properties

C_{60} may be reversibly reduced by up to six electrons.[37] Molecular orbital calculations predict very low lying triply degenerate LUMOs.[38,39] The electron affinity of C_{60} can be explained qualitatively by considering its numerous pyracylene units, which upon receiving 2 electrons could go from an unstable $4n$ π-system to a stable aromatic $4n+2$ π system.[40] Moreover, the formation of sp^3-like anionic centers may lower the energy of the somewhat strained fullerene surface of bent sp^2 carbons.

2.3 Light absorption

Fullerenes absorb strongly in the UV and moderately in the visible regions of the spectrum (Fig. 1).[41–43] The UV absorption of C_{60} in hexanes is characterized by intense broad absorption bands at 211, 256, and 328 nm. The band at 211 has a shoulder at 227 nm and that at 328 nm has several distinguishable shoulders at 365,

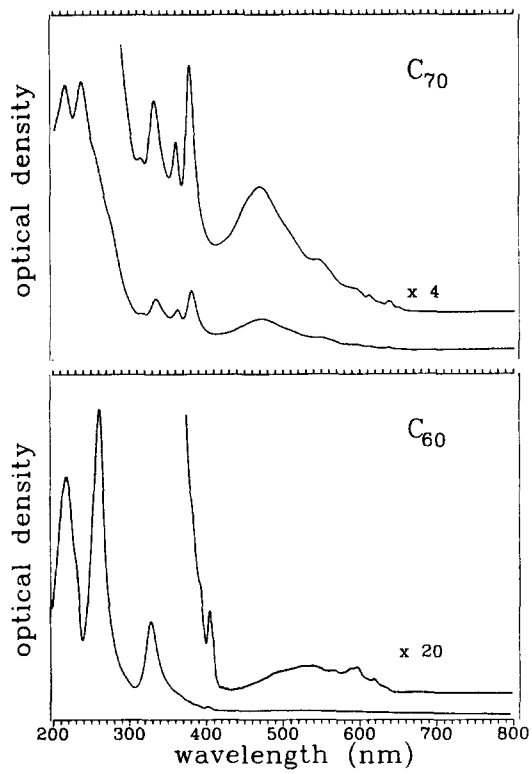


Figure 1. UV–vis absorption spectra of C_{60} and C_{70} in hexanes. Reprinted with permission from 42. Copyright 1990 American Chemical Society.

377, 391, and 396 nm extending into the visible region. These shoulders are indicative of vibrational structure. A minor peak appears in the visible at 404 nm, which descends to a minimum from 420 to 440 nm before rising again to relatively weak absorption bands of varying width and intensity from 440 to 635 nm corresponding to symmetry-forbidden transitions. The visible absorption minimum at around 430 nm makes dilute C_{60} solutions purple. In general, the UV absorption of C_{60} derivatives is similar to that of C_{60}.

UV absorption of C_{70} (see Fig. 1) shows two intense peaks at 215 and 236 nm, the latter containing three shoulders as it approaches a minimum at around 310 nm. Three peaks of varying medium intensity appear at 331, 359, and 378 nm. These are followed by another lower absorption minimum from about 400 to 430 nm. The spectrum then rises slowly, forming a broad peak with a maximum at 469 nm, which slowly decreases to zero by 650 nm after passing through a series of smaller peaks and shoulders. The color of C_{70} in dilute solutions is orange–red.

Singlet excited states of C_{60} ($^1C_{60}$) and C_{70} ($^1C_{70}$) are initially formed upon light excitation.[44–46] Very little fluorescence is observed for either C_{60} ($\Phi_f \approx 10^{-5}$–10^{-4}; where Φ_f is the quantum yield of fluorescence—the ratio of photons which fluoresce to photons absorbed) or C_{70} ($\Phi_f = 10^{-4}$ at 77 K).[46] The lifetime (τ) of $^1C_{60}$ is $\sim$1.3 ns, while that of $^1C_{70}$ is $\sim$700 ps.[46] The singlet states were characterized by recording their respective transient absorption spectra and then monitoring the transient decay.[46]

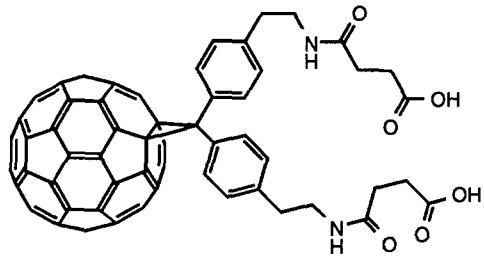
The predominant decay mode of fullerene singlets is intersystem crossing to triplets. Quantum yields of triplet formation (Φ_t) for $^3C_{60}$ and $^3C_{70}$ are almost unity.[44–46] This is explained by the relatively large spin orbit coupling of C_{60} due to its spherical geometry.[47] Transient absorption spectra for $^3C_{60}$ and $^3C_{70}$ were also measured and their lifetimes were determined spectroscopically and photothermally.[46] The triplet lifetime of $^3C_{60}$ in solution is 130 μs and that of $^3C_{70}$ is ca. 2.2 ms.[46] Additionally, fullerene triplets can be formed indirectly using triplet sensitizers such as acridine and anthracene, and are quenched by triplet quenchers such as rubrene, tetracene, and ground state triplet oxygen ($^3O_2$).[44,45] If O_2 is present, $^3C_{60}$ efficiently (almost 100%) sensitizes formation of $^1O_2$.[44] The behavior of $^3C_{70}$ is similar to that of $^3C_{60}$, although the efficiency of $^1O_2$ formation is slightly smaller ($\sim$81%).[44–46] $^1O_2$ is a highly reactive form of O_2 that can damage biomolecules such as DNA. The efficient generation of $^1O_2$ by photoexcited C_{60} and C_{70} makes fullerenes candidates for DNA cleavage and photodynamic therapy (*vide infra*).

3. Biological Applications

3.1 Enzyme inhibition

In 1993 Tokuyama et al.[48] synthesized and tested a series of fullerenes (**5a–b**, shown previously) for photoinduced enzyme inhibition. Fullerene **5a** was found to inhibit cysteine proteinases (m-calpain and papain) and serine proteinases (trypsin, plasmin, and thrombin) when exposed to low-energy light. The same derivative was inactive against cathepsin D, acyl-CoA cholesterol acyltransferase, diacylglycerol acyltransferase, HIV-reverse transcriptase (RT), and the sterol biosynthesis enzymes. The mechanism for inhibition was proposed to involve $^1O_2$.

Based on molecular modeling, Friedman et al. anticipated that a C_{60} molecule should fit nicely into the hydrophobic cavity of the protease specific for the human immunodeficiency virus HIV-1.[21] Using the program DOCK3 they were able to fit a minimized structure of C_{60} into the enzyme active site. Good van der Waals interactions with the hydrophobic surface resulted when the C_{60} was squarely in the center of the cavity. Calculations indicated that 298 Å^2 of hydrophobic surface was removed from exposure to solvent upon binding of the fullerene. The free energy of binding was estimated to be 8–12 kcal/mol, corresponding to a dissociation constant K_d of 10^{-6}–10^{-9} M. In this model, interaction of the catalytic aspartate residues of the enzyme with the fullerene was not specifically taken into account. A computer-minimized inclusion complex of the water-soluble C_{60} derivative **6**, prepared in three steps from C_{60} by Sijbesma et al.,[22] again positions the fullerene in the center of the hydrophobic cavity at the enzyme active site.[21] Experimentally, fullerene **6** was found to be a competitive inhibitor of recombinant affinity-purified HIV-1 protease (HIVP), with a K_i value of 5.3 μM.[21] For comparison,



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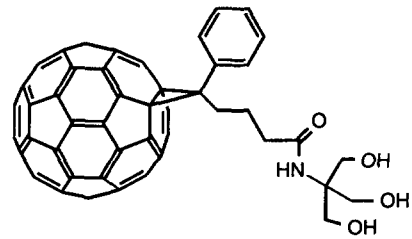

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the best peptide-based protease inhibitors are effective in the subnanomolar range, while nonpeptide inhibitors are effective in the high nanomolar range.

3.2 Antiviral activity

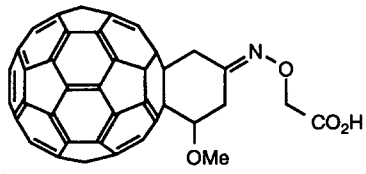
Fullerene derivative **6** was tested by Schinazi et al.[23] for antiviral activity in cells acutely and chronically infected with HIV-1 and in cell-free systems. In human peripheral blood mononuclear cells (PBMC) infected with HIV type IIIA-I, the antiviral activity (EC_{50}) of **6** was found to be 7.3 ± 5.9 μM. Compound **6** was also active against chronically infected H9 cells (EC_{50} 10.8 ± 8.2 μM) and human PBMC acutely infected with HIV-2_{ROD} (EC_{50} of 5.5 ± 3.8 μM). While the anti-AIDS drug 3′-azido-3′-deoxythymidine (AZT) has significantly greater activity against acutely infected cells (EC_{50} 0.003–0.004 μM), it is not active in chronically infected H9 cells. When cell-free HIV-1 was incubated with **6** at concentrations of 5–25 μM, virus infectivity was reduced by more than 95% relative to controls, demonstrating that **6** interacts directly with the virus. Since agents used to treat viruses frequently lead to the development of drug resistant viral strains, fullerene **6** was tested against AZT-susceptible HIV-1_{H112-2} as well as AZT-resistant HIV-1_{G910-6} in acutely infected primary human lymphocytyes. The activity of **6** was the same in both cases, indicating no cross-resistance between **6** and AZT. This suggests that combination therapy using water-soluble fullerene derivatives and AZT might be fruitful.

The anti-HIV activity of **6** was also shown by Schinazi et al. to be selective, since **6** did not bind to $CD4^+$ receptors on the surface of human lymphocytes, which are primary targets for the HIV virus, nor with viral glycoprotein gp 120. Thus, **6** has potent and selective activity against HIV-1 in both acutely and chronically infected cells due to direct interaction with the virus, as well as inhibition of the HIV-1 protease and reverse transcriptase. Furthermore, no cytotoxicity was observed with **6** up to 100 μM in uninfected slowly dividing PBMC or rapidly dividing H9, Vero or CEM cells under conditions where AZT is cytotoxic in all but the first cell line. In addition, when **6** was administered intraperitoneally to mice in doses up to 50 mg/kg/day for 6 days, all the animals (18 were given **6** in various dosages + 18 controls) steadily gained weight and none died up to 2 months after initial treatment.[25] Schinazi et al. concluded that other water-soluble fullerenes that behave similarly but with even greater potency might be discovered. Such compounds might find use, for example, in inactivating HIV in blood and blood products as well as genital secretions.[25]

More recently, the tris-hydroxymethyl methanofullerene derivative **7** was prepared and evaluated for anti-HIV activity by Schinazi, Wudl, and co-workers using the same assays as described above for **6**.[27] Compound **7** was slightly more active than **6** against HIV-1_{LAI} in human peripheral blood mononuclear cells (EC_{50} 2.5 μM versus 7.5 μM) and demonstrated no toxicity up to 100 μM in two human and one monkey cell system (PBM, Vero, and CEM cells).

Eleven additional C_{60} derivatives synthesized at New York University by Wilson, Schuster, and co-workers were tested as DMSO/water emulsions against human PBMC infected with HIV-1, strain LAI.[49] All but one of these showed antiviral activity (EC_{50}) in the low micromolar range. The three most active were compounds **8**, **9**, and **10** (EC_{50} 0.88, 2.2, and 2.9 μM, respectively). These and all but one of the other test compounds demonstrated no toxicity in PBM and Vero cells ($IC_{50} > 100$ μM). Although the mechanism of anti-HIV activity of these compounds has not been established using cell-free assays, it appears that anti-HIV activity and low toxicity seem to be general properties of many types of C_{60} derivatives, although the pattern of activity may vary from compound to compound. Since compounds **8** and **9** are chiral, in contrast to **6** and **7**, it will be possible to determine the enantiospecificity of the anti-HIV activity of **8** and **9** in both cell-based and cell-free assays. The presence or absence of such enantiospecifity in these assays will shed some light on the nature of the interactions responsible for the anti-HIV activity. It will also be interesting to see if these fullerenes have activity against other viruses.

Finally, two C_{70} derivatives (**11** and **12**) prepared by Wilson and co-workers were also evaluated by Schinazi for anti-HIV activity.[49] Models analogous to those used by Friedman et al.[22] indicated that C_{70} fits in the HIVP hydrophobic cavity as well as or better than C_{60}. Preliminary data indicated lower anti-HIV activity for **11** and **12** than for analogous C_{60} derivatives, but the low solubility of these compounds in DMSO may be a contributing factor. It will be necessary to prepare and test C_{70} derivatives with greater solubility in DMSO to resolve this question.



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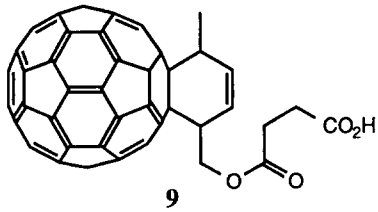

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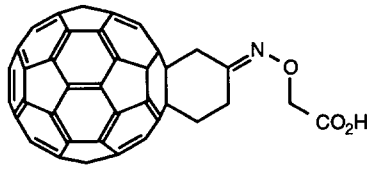

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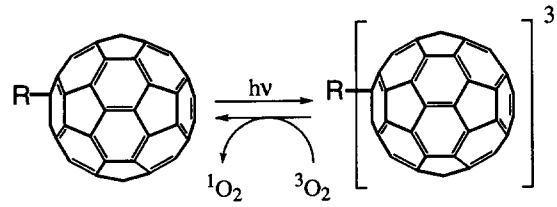
3.3 DNA cleavage

Compounds **5a** and **5c** were found to cleave DNA when incubated with supercoiled pBR322 DNA and then irradiated with light.[48] Compound **5c** was shown to specifically cleave a 182 base-pair fragment at guanine residues upon exposure to light. It is presumed that the mechanism for scission of the oligonucleotides involves photoexcitation of the fullerene portion followed by sensitized formation of $^1O_2$, regenerating ground state fullerene, and interaction of the reactive $^1O_2$ with the oligonucleotide. An overview of the proposed mechanism for DNA cleavage and photodynamic therapy is shown in Figure 2.

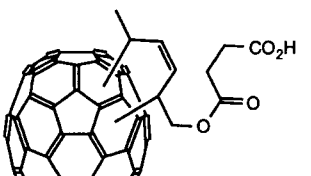


R = Single strand of DNA (DNA Cleavage)
or cancer cell (Photodynamic Therapy)

Figure 2. Applications of C_{60} mediated formation of $^1O_2$. First, $^3C_{60}$ is formed by photoexcitation (100% efficient). Second, $^1O_2$ is generated by the fullerene as a result of sensitization of $^3O_2$ by $^3C_{60}$ as the fullerene decays back to the ground state. Finally, either cleavage of a DNA strand or destruction of a cancer cell could occur as a result of interaction with $^1O_2$.

Boutorine et al. later described the synthesis of a fullerene-oligonucleotide (**13**) that could bind single-stranded DNA, double-stranded DNA, and double-stranded DNA with a hairpin to form a duplex, triple helix, and triple helix with hairpin, respectively.[50] The addition of the fullerene to the complementary strand did not appear to hinder the formation of the duplex or triple helices. In each case the conjugate was cleaved specifically at guanine residues proximal to the fullerene moiety upon exposure to light. Once again, $^1O_2$ was suspected to be the cleaving species.

In a related study, An et al. found that another fullerene-oligonucleotide (**14**) also cut DNA specifically at guanine residues located near the fullerene terminus of the oligonucleotide.[51] They investigated the possible intermediacy of $^1O_2$ by comparing the reactivity of the fullerene-oligonucleotide with a similarly linked eosin-oligonucleotide, which is known to sensitize $^1O_2$ formation. A favorable environment for $^1O_2$ formation was provided by performing the experiment in D_2O instead of water, because $^1O_2$ has a significantly longer lifetime in D_2O than in water.[52] Eosin-oligonucleotide cleavage was more efficient in D_2O, while the fullerene-oligonucleotide exhibited the same rate in both solvents. Also, sodium azide (a $^1O_2$ quencher) was found to inhibit the eosin-oligonucleotide DNA cleavage, but not the fullerene-oligonucleotide cleavage. These results suggest that the fullerene-oligonucleotide cleavage mechanism does not involve $^1O_2$, but may involve a direct interaction



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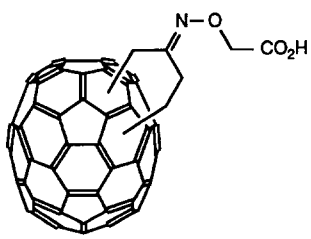

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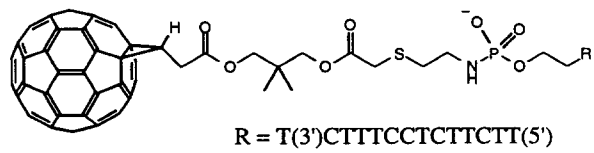
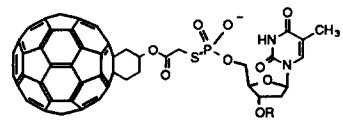


R = T(3')CTTTCCTCTTCTT(5')

13



R = C(5')TAACGACAATATGTACAAGCCTAATTGTGTAGCATCT(3')

14

between the guanosine residues and the fullerene, such as a electron transfer.

3.4 Photodynamic therapy

As mentioned above, C_{60} and C_{70} are efficiently converted to their triplet states upon UV and visible irradiation and C_{60} and C_{70} triplet states readily convert $^3O_2$ to $^1O_2$.[44–46] It was suggested that fullerenes had a 'strong potential for photodynamic damage to biological systems'.[44]

Fullerenes **5a** and **5c** were shown to be cytotoxic upon incubation with HeLa S3 cells and irradiation with light.[48] The same mixtures of fullerenes and cells was unaffected when not exposed to light. Compound **5b** was not cytotoxic, even when irradiated, possibly due to its relatively low water-solubility compared with **5a** and **5c**.[48] A reference compound lacking the fullerene moiety but possessing a side chain similar to that of **5a** and **5c** was inactive when incubated with the cells and exposed to the same light source. These results can be explained by interaction of $^1O_2$ with the cells or direct interaction between the fullerene excited state and the cells.

Li et al. reported that when a mixture of liposome-encapsulated C_{60} and human cervix cancer cells was irradiated for a short period of time, the irradiated cells showed a twofold *increase* in proliferation relative to the unirradiated cells.[53] The authors stated that "this phenomenon is in accord with our experience in laser irradiation experiments with HPD (hematoporphyrin derivative) [in] that the cancer cell will be killed only [under] condition[s] [such] that the laser power is above some threshold [for a] suitable duration of irradiation time, otherwise it will have promoted cell growth".[53]

Recently, Nakajima et al. reported that C_{60} functionalized with polyethylene glycol units (to enhance solubility) was cytotoxic when irradiated with visible light but not in the dark.[54] These fullerene derivatives were not well characterized due to their instability. It was postulated that two separate derivatives were made, depending on the reaction conditions used. The first type supposedly consists of polyethylene glycol attached to the fullerene via a nitrogen–carbon bond, prepared by reaction of an amino terminal polyethylene glycol with C_{60}. The second type, from reaction of 1,2-diaminoethane with C_{60}, supposedly formed a cyclic diamine adduct. Addition of polyethylene glycol with a terminal carboxylic acid could protonate the amino units and form ionic bonds between the C_{60} and the polymer.

Interestingly, they showed that cytochrome C is reduced when it is irradiated with the fullerene derivatives. This could imply generation of superoxide. When superoxide dismutase (SOD) was also added, reduction of cytochrome C was suppressed. Cytotoxicity as a function of SOD added was also determined. The results showed "no clear change in the cytotoxicity ... even upon addition of SOD as high as 3000 unit/mL".[54] The authors further stated "this clearly indicates that the superoxide produced by light irradiation was not converted by SOD".[54] However, when SOD, cytochrome C and the C_{60} derivatives were added together, apparently without cells, the SOD suppressed cytochrome C reduction. This apparent discrepancy was justified by the theory that the C_{60} adduct might be taken up by the cells while the SOD might not be.[54] However, the reverse could also be true. The authors further postulated that $^1O_2$ production by fullerenes may occur indirectly via superoxide.[54]

3.5 Electron transfer

As discussed previously, C_{60} readily accepts up to six electrons,[37] due to its low lying, triply degenerate LUMOs.[38,39] This has led to interest in the electron-transfer capabilities of fullerenes in biological systems. In one such report by Hwang and Mauzerall in 1993,[15] it was reported that C_{70} could be used to mediate electron transport through a lipid bilayer system in which the C_{70} was imbedded in the membrane.

They formed a C_{70}-containing lipid bilayer in a 1.5 mm diameter hole of a thin Teflon sheet. On one side of the lipid bilayer was an aqueous solution containing ascorbate, an electron donor, and on the other side, an aqueous compartment containing anthraquinone 2-sulphonate, an electron acceptor. They then positioned an electrode in each of the aqueous compartments. Upon irradiation with light, after removal of oxygen, a current was observed between the aqueous compartments which showed no decrease for the first 2 min and then slowly decayed to zero by 30 min. In the absence of C_{70}, no current was observed, nor was current observed if oxygen was not removed from the bilayer.

They suggested that electron transport proceeds via "electron hopping among fullerene molecules, possibly in the form of aggregates".[15] However, they did not rule out the possibility of reduction of C_{70} molecules near the boundary and diffusion of the C_{70} radical anions across the bilayer, followed by release of the electron on the other side of the membrane (see Fig. 3).

Prior to their trans-membrane electron transport study, Hwang and Mauzerall reported intermolecular electron transfer between porphyrins and fullerenes.[16] This work was carried out by measuring the photovoltage between fullerenes imbedded in a lipid bilayer and porphyrins attached to the bilayer–water interface upon irradiation with light.

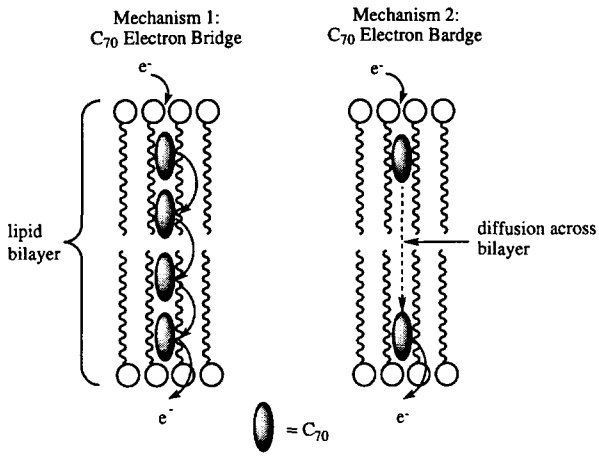
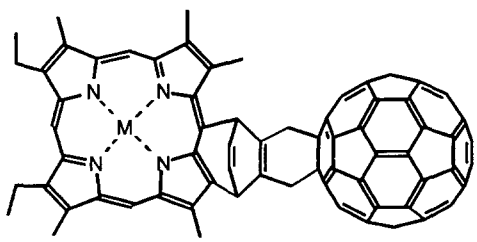


Figure 3. Trans-membrane electron transport mediated by C_{70}. The mechanism could either involve electron hopping along a bridge of C_{70} molecules within the membrane or reduction of individual C_{70} molecules, followed by diffusion to the other side of the bilayer and release of the electron.

Gust and coworkers reported the preparation of a fullerene–porphyrin dyad (**15**).[55] When the porphyrin moieties of both the Zn-complexed and free base dyads are selectively irradiated in toluene solvent, the porphyrin singlet excited state is formed. Intramolecular energy transfer is then observed from the singlet porphyrin moieties to the C_{60} moiety, generating the C_{60} singlet. For the Zn-complexed dyad, the $^1C_{60}$ unit accepts an electron from the porphyrin, forming the C_{60} radical anion and porphyrin radical cation. For the free base porphyrin in toluene, the excited $^1C_{60}$ intersystem crosses to $^3C_{60}$ which sensitizes formation of $^1O_2$. In the more polar solvent benzonitrile, the $^1C_{60}$ portion of the free base dyad undergoes electron transfer to form the C_{60} radical anion and porphyrin radical cation.

In a similar study, Imahori et al. reported the photophysical properties of porphyrin C_{60} dyad **16**.[56] In agreement with the previous study,[55] quenching of both the singlet excited Zn-complexed and free base porphyrins by the C_{60} moiety in THF was observed, as well as electron transfer from the Zn-complexed porphyrin to $^1C_{60}$. This was not observed in the free base dyad. They further indicated that there is little difference between the visible absorption spectra of the dyads and the untethered porphyrins, except for minor



M = Zn and H_2

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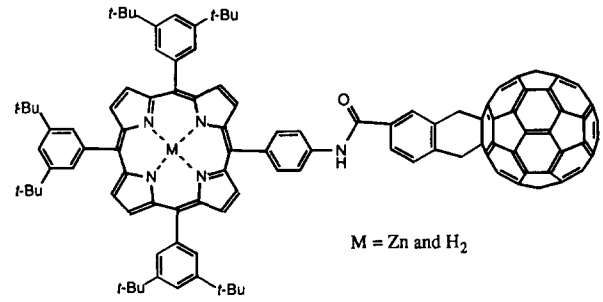

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peaks attributed to visible absorption by the C_{60} portion. Thus, there appears to be no significant ground state interaction between the two chromophores.

Chiang and co-workers have reported some interesting biological effects of water-soluble polyhydroxylated C_{60} (**3**) in whole blood samples, which they attribute to scavenging of intermediate free radicals.[57,58] The fullerenols were prepared via nitronium-activated nucleophilic attack on C_{60}, through reaction of C_{60} with nitronium tetrafluoroborate and arenecarboxylic acids in methylene chloride, followed by alkaline hydrolysis. It is presumed that initial addition of nitronium ions to C_{60} activates the fullerenes to nucleophilic attack by the arenecarboxylate ion. From ^1H NMR spectroscopy and elemental analysis, the chemical composition of the adducts from *p*-bromobenzoate indicated an average of 4–5 *p*-bromobenzoates and 13–15 hydroxyl groups per C_{60} molecule, corresponding to 17–20 hydroxyl groups per C_{60} after alkaline hydrolysis. Fullerols were also produced by reaction of C_{60} or C_{70} with fuming sulfuric acid followed by addition of potassium nitrate. Mass spectrometry indicated the presence of numerous hydroxylated fullerenes, while ^{13}C NMR spectroscopy (solid state and in D_2O solution), suggested the presence of several hemiacetal moieties as well as tertiary hydroxyl groups.

It was anticipated that this mixture of polyhydroxylated fullerenes might act as a water-soluble free radical trap, based upon the known susceptibility of C_{60} to radical attack. This was explored in a study of chemiluminescence from blood samples treated with lucigenin. In the absence of whole blood, only minimal chemiluminescence from lucigenin was observed. A moderate increase in chemiluminescence was observed when whole blood from healthy human subjects was added, attributed to reaction of lucigenine with superoxide radicals in the blood samples; this emission was suppressed in the presence of superoxide dismutase. When blood from male and female patients with gastric cancer (number of patients not given) was used, a high intensity of chemiluminescence upon addition of lucigenine was observed. When the whole blood from the same patients suffering with cancer was pretreated with the fullerenol mixture at a concentration of 60 μg/mL, a sharp decrease in chemiluminescence was observed. This was assumed to be the result of inter-

ception of superoxide radicals, as seen with superoxide dismutase. Assuming the fullerenols are acting as radical traps, the scavenging efficiency at this concentration is ~85%.

Further studies are needed to more fully probe the mechanism of action of this mixture of fullerenols. Since polyhydroxy compounds in general show antioxidant activity, the uniqueness of the behavior of these fullerenols remains to be established. Nonetheless, if these interesting results can be replicated, generalized and extended to larger patient populations, it is possible that water-soluble fullerenes may have important clinical applications in treatment of conditions where production of free radicals occurs in response to invading pathogens.

3.6 Miscellaneous applications

Tsuchiya et al. recently investigated the effect of C_{60} on differentiation and proliferation of rat embryo cells.[20] They solubilized C_{60} by interaction with poly(vinylpyrrolidone) (PVP),[19] and found that C_{60} promoted cell differentiation by a factor of 3.2 over control cells with and without PVP. However, the proliferation of the cells was slowed relative to the controls. PVP is not an ideal solubilizing macromolecule because it promotes differentiation and inhibits proliferation.[20] Yamakoshi et al. showed that PVP-solubilized C_{60} had no effect on hemolysis of sheep red blood cells.[19] The hemolytic properties of C_{60} have also been investigated by Baierl et al.[59]

A new technique for studying many types of biological properties of fullerenes was recently reported by Richmond and Gibson.[60] The technique involves vapor deposition of fullerenes (a mixture predominantly of C_{60} and C_{70} derived from unrefined soot) on various types of surfaces, which were then used to support growth of mammalian cells and attachment of DNA and proteins. Cells grew only on fullerene-coated glass, consistent with the finding that fullerene coating over glass provides a somewhat more hydrophilic surface compared with the same coating over polystyrene, due to the underlying mono- and divalent cations in the glass. In initial studies, Chinese hamster ovary AA8 cells were seeded onto fullerene-coated or partially masked glass surfaces (slides and culture dishes), and cell growth to give a monolayer then proceeded after exposure to appropriate media. Exposure of the cells in an oxygen atmosphere to a visible wavelength light beam with no UV and only 10% IR content led to damage in cells deposited on fullerene-coated surfaces, but not to cells deposited directly on the glass surface. Membrane damage to the cells was measured by the uptake of trypan blue stain. Cell damage, which did not take place in the absence of oxygen, was dependent on light intensity (J/cm^2). Although a photodynamic effect of the fullerenes is clearly implicated, there was no direct experimental proof that singlet oxygen ($^1O_2$) was involved in these experiments. However, given the known efficacy of fullerenes as $^1O_2$ sensitizers, formation of $^1O_2$ under the reaction conditions is highly likely.

It was also observed that DNA and protein bound to different surfaces in the following order: nitrocellulose > fullerene-coated glass > glass > polystyrene. The attachment of biogenic factors to the fullerene surface was rationalized in terms of weak π-interactions, as in adsorption of such materials to the surface of activated carbon.

These findings open up the possibility of studying membrane-related drug interactions and membrane-based events that direct processes such as signal transduction, cell fusion and differentiation on fullerene-coated surfaces after adsorption of appropriate biogenic factors. Since the photodynamic effect observed here clearly results in a significant increase in cell membrane permeability, the entry of various agents (e.g., transfecting DNA, antibodies, proteins and other intermediates) into cells could be manipulated by illuminating cells on fullerene surfaces.

Wilson et al. synthesized fullerene steroids **17–20** using thermal cycloadditions to form **17** and **18** and [2+2] photocycloadditions to form **19** and **20**.[61] In a preliminary assay **18** inhibited the binding of ^3H estradiol to estrogen receptors present in calf uterus cytosol. Rubin and co-workers have also synthesized steroidal fullerenes **21** and **22**[62] and fullerene amino acids **23** and **24**,[63] but did not report biological activity of these compounds. Wudl and co-workers have also reported the synthesis of a fullerene steroid **25**.[64] Vasella, Diederich, and co-workers have reported the synthesis of fullerene sugars **26** and **27**.[65]

4. Metabolism, Excretion and Toxicity

Besides getting a potential fullerene drug to the target cell, an important issue for development of pharmaceuticals based on fullerene units involves metabolism and excretion. As alluded to previously, a labeled version of **5a** (^{14}C **5a**) has recently been tested for biological behavior in rats by Yamago et al.[30] When ^{14}C **5a** was fed orally to the rats, minimal absorption occurred as most of the compound was excreted in the feces. However, upon intravenous injection, most of the compound was rapidly (within 1 h) transported to the tissues. Surprisingly, after 1 week only 5.4% was fecally excreted and less than 2% remained in the organ tissues; the remainder was distributed in the skeletal muscle and hair. This raises questions regarding prolonged exposure to fullerene drugs. Acute toxicity of ^{14}C **5a** was low. Since the fullerenes are rather quickly carried to the tissues, it may be possible to use them as carriers of highly polar drugs to certain hydrophobic sites and tissues such as the liver.[30] However, while no toxic effects due to accumulation have been reported thus far, deposition of C_{60} is a negative factor in potential biomedical applications. Also noteworthy is the observation that ^{14}C **5a** was able to cross the blood–brain barrier.[30]

A very recent study involving injection of suspensions of ^{14}C-labeled C_{60} and the more polar ammonium salt

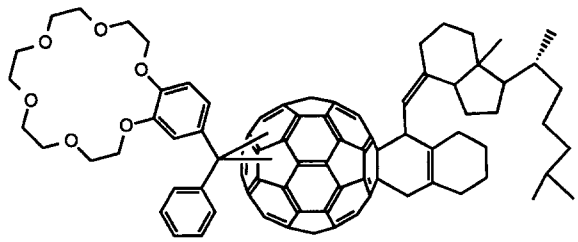
26 in female Sprague–Dawley rats indicates that the fullerenes rapidly clear from circulation and most of the radioactivity appears in the liver (see Table 1).[66]

These data contrast with results of Yamago et al.,[30] particularly with regard to crossing the blood–brain barrier.

Scrivens et al.[66] have also studied cellular uptake of underivatized C_{60} by preparing ^{14}C-doped C_{60} as a fine aqueous suspension, thought to be composed of small clusters of C_{60} molecules, and monitoring its uptake by cells.[8] This suspension was then used to treat human keratinocytes. The radioactive C_{60} was apparently taken up by the cells; however, it is unclear whether or not the C_{60} was merely associated with the surface of the cells, was incorporated into the lipid bilayer, or was taken into the cells. Unlabeled C_{60} did not appear to affect the proliferation rate of cultured human keratinocytes and human fibroblasts.[7,8] ^{14}C-doped C_{60} has also been prepared in our labs.[67]

One interesting result that is consistent with all the above studies is that fullerenes accumulated in the liver do not appear to be metabolized. While most organic compounds in the liver are oxidized, conjugated and then excreted, the fullerenes and derivatives studied so far remain apparently unchanged in the liver.

A direct by relevant study of this problem was recently presented at the Pacifichem-96 meeting.[68] They reported the conversion of C_{60} into its epoxide chemically, but not with rat liver microsomes under conditions known to oxidize polynuclear aromatic hydrocarbons. Taken together, all of these results suggest that, perhaps not unexpectedly, the fullerene



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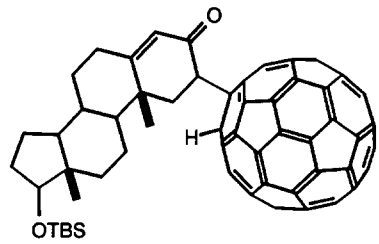

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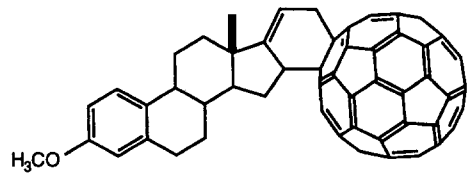

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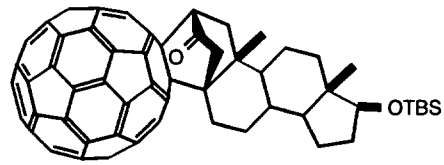

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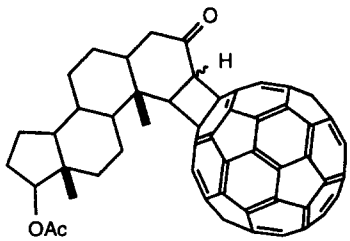

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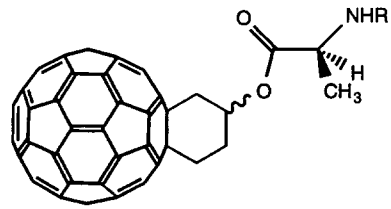
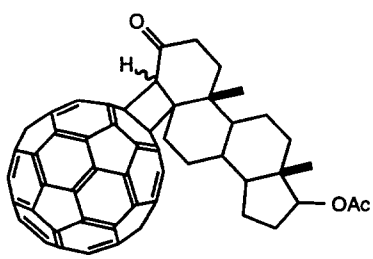


23 R = *t*-Boc, Cbz, or H



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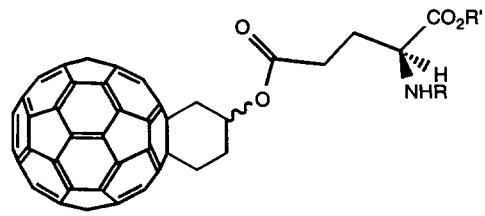
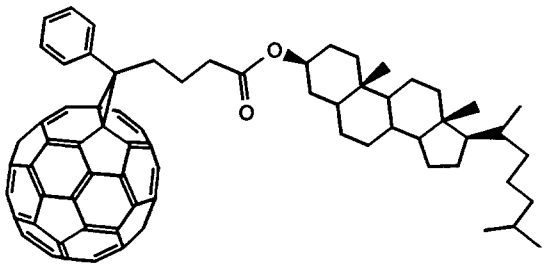


24a R = *t*-Boc, R' = Bn
24b R, R' = H



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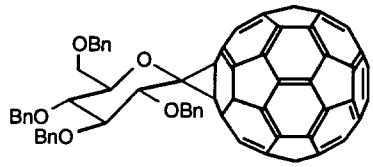

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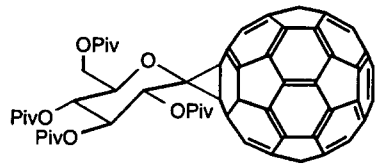

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portion of C_{60} and its derivatives is quite stable to metabolic attack.

A few additional studies concerning fullerene toxicity have appeared. Nelson et al. topically administered C_{60} to skin in animal trials to determine possible health risks.[69] Questions had arisen as to the potential carcinogenicity of C_{60}. At the dosages used it was found that there were no deleterious effects of the topically administered C_{60} on mouse epidermal DNA synthesis or induction of ornithine decarboxylase activity after 72 h. Furthermore, repeated administration of the fullerene for up to 24 weeks did not result in benign or malignant skin tumor formation. As mentioned previously, Schinazi et al. reported that no toxicity was observed when **6** was given intraperitoneally to mice for 6 days (up to 50 mg/kg/day).[25] None of the mice died within 2 months of the initial treatment, although some initial weight loss was noted.[25] Yamago et al. tested the toxicity of a methanofullerene version of **5a** through one intraperitoneal injection (200–500 mg/kg). Although the mice showed some initial writhing, stretching, and weight loss, they all survived for 1 week.[30] Zakharenko et al. estimated the genotoxicity of fullerene and fullerol compounds by assaying for mutations in *Escherichia coli* and larvae,[70] and found that C_{60} and a polyhydroxylated C_{60} derivative were essentially nongenotoxic.

Very recently, Moussa et al. injected large amounts of micronized C_{60} into Swiss mice, and found that crystals of C_{60} collected in the spleen and liver.[13] Photographs of granular deposits of C_{60} *within* the cells were taken. These pictures clearly demonstrate the ability of C_{60} (unfunctionalized) to pass through cellular membranes (see Fig. 4).

Table 1. Distribution of radiolabeled C_{60} and ammonium salt **28** in tissues after intravenous injection. Reprinted with permission from J. M. Tour[66]

	$^{14}C_{60}$	$^{14}C_{60}$-Ammonium salt
Adrenals	<0.1	<0.1
Bladder	<0.1	<0.1
Brain	<0.1	<0.1
Eyes	<0.1	<0.1
Fat	0.3	2.1
Heart	<0.1	0.1
Kidney	<0.1	0.1
Liver	81.6	46.0
Lungs	1.3	126.2
Muscle	0.9	12.9
Red blood cells	3.3	11.4
Plasma	<0.1	2.8
Skin	0.7	9.0
Spleen	3.8	1.4
Urine	0.2	<0.1

Figure 4. Picture of crystals of C_{60} (marked by arrows) in a liver fat-storing cell. Reprinted with permission from 13. Copyright 1996 Marcel Dekker, Inc.

5. Summary and Conclusions

Thus far, development of applications of fullerenes in biology has been hampered by the poor water solubility of fullerenes. In spite of such concerns, fullerenes have proved useful for a wide variety of biological applications. As derivatized and underivatized fullerenes continue to become increasingly available, additional applications and further development of those discussed in this article will invariably follow.

Acknowledgment

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References

1. (a) Hill, C. L. *Proc. Electrochem. Soc.* **1994**, *94-24*, 659; (b) Hill, C. L. *Proc. Electrochem. Soc.* **1995**, *95-10*, 683.

2. Ruoff, R. S.; Tse, D. S.; Malhotra, R.; Lorents, D. C. *J. Phys. Chem.* **1993**, *97*, 3379.

3. Sivaraman, N.; Dhamodaran, R.; Kaliappan, I.; Srinivasan, T. G.; Vasudeva Rao, P. R.; Mathews, C. K. *Proc. Electrochem. Soc.* **1994**, *94-24*, 156.

4. Scrivens, W. A.; Cassell, A. M.; Kinsey, K. E.; Tour, J. M. *Proc. Electrochem. Soc.* **1994**, *94–24*, 166.

5. Beck, M. T.; Mándi, G.; Kéki, S. *Proc. Electrochem. Soc.* **1995**, *95-10*, 1510.

6. Andrievsky, G. V.; Kosevich, M. V.; Vovk, O. M.; Shelkovsky, V. S.; Vashchenko, L. A. *Proc. Electrochem. Soc.* **1995**, *95–10*, 1591.

7. Scrivens, W. A.; Tour, J. M.; Creek, K. E.; Pirisi, L. *J. Am. Chem. Soc.* **1994**, *116*, 4517.

8. Scrivens, W. A.; Tour, J. M.; Creek, K. E.; Pirisi, L. *Proc. Electrochem. Soc.* **1994**, *94-24*, 676.

9. Andersson, T.; Nilsson, K.; Sundahl, M.; Westman, G.; Wennerstrom, O. *J. Chem. Soc. Chem. Commun.* **1992**, 604.

10. Sundahl, M.; Andersson, T.; Nilsson, K.; Wennerstrom, O.; Westman, G. *Synth. Met.* 1993, *55–57*, 3252.

11. Wilson, S. R.; Lu, Q.; Yang, Y.; Echegoyen, L. *Tetrahedron Lett.* submitted.

12. Atwood, J. L.; Koutsantonis, G. A.; Raston, C. L. *Nature* **1994**, *368*, 229.

13. Moussa, F.; Trivin, F.; Céolin, R.; Hadchouel, M.; Sizaret, P.-Y.; Greugny, V.; Fabre, C.; Rassat, A.; Szwarc, H. *Fullerene Sci. Technol.* **1996**, *4*, 21.

14. Garaud, J. L.; Janot, J. M.; Miquel, G.; Seta, P. *J. Membr. Sci.* **1994**, *91*, 259.

15. Hwang, K. C.; Mauzerall, D. *Nature* **1993**, *361*, 138.

16. Hwang, K. C.; Mauzerall, D. *J. Am. Chem. Soc.* **1992**, *114*, 9705.

17. Bensasson, R. V.; Bienvenue, E.; Dellinger, M.; Leach, S.; Seta, P. *J. Phys. Chem.* **1994**, *98*, 3492.

18. Hungerbuhler, H.; Guldi, D. M.; Asmus, K.-D. *J. Am. Chem. Soc.* **1993**, *115*, 3386.

19. Yamakoshi, Y. N.; Yagami, T.; Fukuhara, K.; Sueyoshi, S.; Miyata, N. *J. Chem. Soc. Chem. Commun.* **1994**, 517.

20. Tsuchiya, T.; Yamakoshi, Y. N.; Miyata, N. *Biochem. Biophys. Res. Commun.* **1995**, *206*, 885.

21. Friedman, S. H.; DeCamp, D. L.; Sijbesma, R. P.; Srdanov, G.; Wudl, F.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6506.

22. Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; DeCamp, D. L.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510.

23. Schinazi, R. F.; Sijbesma, R.; Srdanov, G.; Hill, C. L.; Wudl, F. *Antimicrob. Agents Chemother.* **1993**, *37*, 1707.

24. Friedman, S. H.; Wudl, F.; Rubin, Y.; Kenyon, G. L. *Proc. Electrochem. Soc.* **1994**, *94-24*, 662.

25. Schinazi, R. F.; McMillan, A.; Juodawlkis, A. S.; Pharr, J.; Sijbesma, R.; Srdanov, G.; Hummelen, J.-C.; Boudinot, F. D.; Hill, C. L.; Wudl, F. *Proc. Electrochem. Soc.* **1994**, *94-24*, 689.

26. Triggle, D. J. *Chemtracts: Org. Chem.* **1994**, *7*, 57.

27. Schinazi, R. F.; Bellavia, C.; Gonzalez, R.; Hill, C. L.; Wudl, F. *Proc. Electrochem. Soc.* **1995**, *95-10*, 696.

28. Chiang, L. Y.; Wang, L.-Y.; Swirczewski, J. W.; Soled, S.; Cameron, S. *J. Org. Chem.* **1994**, *59*, 3960.

29. Wudl, F.; Hirsch, A.; Khemani, K. C.; Suzuki, T.; Allemand, P. M.; Koch, A.; Eckert, H.; Srdanov, G.; Webb, H. M. *ACS Symp. Ser.* **1992**, *481*, 161.

30. Yamago, S.; Tokuyama, H.; Nakamura, E.; Kikuchi, K.; Kananishi, S.; Sueki, K.; Nakahara, H.; Enomoto, S.; Ambe, F. *Chem. Biol.* **1995**, *2*, 385.

31. Deardon, J. C. In *Comprehensive Medicinal Chemistry*; Hansch, C.; Sammes, P. G.; Taylor, J. G., Eds.; Pergamon: Oxford, 1990; Vol. 4, Section 19.2.

32. Press, M. A.; Unger, S. H. In *Comprehensive Medicinal Chemistry*; Hansch, C.; Sammes, P. G.; Taylor, J. G., Eds.; Pergamon: Oxford, 1990; Vol. 4, Section 21.2.

33. Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley: New York, 1979; Ch. 3.

34. Seiler, P. *Eur. J. Med. Chem.* **1974**, *5*, 473.

35. Young, R. C.; Mitchell, R. C.; Brown, T. H.; Ganellin, C. R.; Griffiths, R.; Jones, M.; Rana, K. K.; Saunders, D.; Smith, I. R.; Sore, N. E.; Wilks, T. J. *J. Med. Chem.* **1988**, *31*, 656.

36. von Geldern, T.; Hoffman, D.; Kester, J.; Nellans, H.; Marsh, K.; Hernandez, L.; Dayton, B.; Calzadilla, S. Presented at the 210th National Meeting of the American Chemical Society, Chicago, IL, August 1995; paper MEDI 35.

37. Xie, Q.; Perez-Cordero, E.; Echegoyen, L. *J. Am. Chem. Soc.* **1992**, *114*, 3978.

38. Haddon, R. C.; Brus, L. E.; Raghavachari, K. *Chem. Phys. Lett.* **1986**, *125*, 459.

39. Haddon, R. C. *Acc. Chem. Res.* **1992**, *25*, 127.

40. Wudl, F. *Acc. Chem. Res.* **1992**, *25*, 157.

41. Krätschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R. *Nature* **1990**, *347*, 354.

42. Ajie, H.; Alvarez, M. M.; Anz, S. J.; Beck, R. D.; Diederich, F.; Fostiropoulos, K.; Huffman, D. R.; Krätschmer, W.; Rubin, Y.; Schriver, K. E.; Sensharma, D.; Whetten, R. L. *J. Phys. Chem.* **1990**, *94*, 8630.

43. Krätschmer, W.; Fostiropoulos, K.; Huffman, D. R. *Chem. Phys. Lett.* **1990**, *170*, 167.

44. Aborgast, J. W.; Darmanyan, A. P.; Foote, C. S.; Diederich F. N.; Rubin, Y.; Diederich, F.; Alvarez, M. M.; Anz, S. J.; Whetten, R. L. *J. Phys. Chem.* **1991**, *95*, 11.

45. Arbogast, J. W.; Foote, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 8886.

46. (a) Foote, C. S. Topics Curr. Chem. **1994**, *169*, 347; (b) Fraelich, M. R.; Weisman, R. B. *J. Phys Chem.* **1993**, *97*, 11145.

47. Allemand, P.-M.; Srdanov, G.; Koch, A.; Khemani, K.; Wudl, F. *J. Am. Chem. Soc.* **1991**, *113*, 2780.

48. Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918.

49. Schuster, D. I.; Wilson, S. R.; Schinazi, R. F. *Bioorg. Med. Chem. Lett.* **1996**, in press.

50. Boutorine, A. S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Hélène, C. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2462.

51. An, Y.-Z.; Chen, C.-H. B.; Anderson, J. L.; Sigman, D. S.; Foote, C. S.; Rubin, Y. *Tetrahedron*, **1996**, *52*, 5179.

52. Merkel, P. B.; Kearns, D. R. *J. Am. Chem. Soc.* **1972**, *94*, 7244.

53. Li, W.; Qian, K.; Huang, W.; Zhang, X.; Chen, W. *Chin. Phys. Lett.* **1994**, *11*, 207.

54. Nakajima, N.; Nishi, C.; Li, F.-M.; Ikada, Y. *Fullerene Sci. Technol.* **1996**, *4*, 1.

55. Liddell, P. A.; Sumida, J. P.; MacPherson, A. N.; Noss, L.; Seely, G. R.; Clark, K. N.; Moore, A. L.; Moore, T. A.; Gust, D. *Photochem. Photobiol.* **1994**, *60*, 537.

56. Imahori, H.; Hagiwara, K.; Akiyama, T.; Taniguchi, S.; Okada, T.; Sakata, Y. *Chem. Lett.* **1995**, 265.

57. Chiang, L. Y.; Lu, F.-J.; Lin, J.-T. *Mater. Res. Soc. Symp. Proc.* **1995**, *359*, 327.

58. Chiang, L. Y.; Lu, F.-J.; Lin, J.-T. *Proc. Electrochem. Soc.* **1995**, *95–10*, 699.

59. Baierl, T.; Drosselmeyer, E.; Seidel, A. *NATO ASI Ser., Ser. H: Cell. Mol. Effects Miner. Synth. Dusts Fibres* **1994**, *85*, 165.

60. Richmond, R. C.; Gibson, U. J. *Proc. Electrochem. Soc.* **1995**, *95-10*, 684.

61. Wilson, S. R.; Lu, Q.; Cao, J.; Zhao, H.; Wu, Y.; Schuster, D. I. *Proc. Electrochem. Soc.* **1995**, *95–10*, 1179.

62. Ganapathi, P. S.; Rubin, Y.; Friedman, S. H.; Kenyon, G. L. *J. Org. Chem.* 1995, *60*, 2954.

63. An, Y.-Z.; Anderson, J. L.; Rubin, Y. *J. Org. Chem.* 1993, *58*, 4799.

64. Hummelen, J. C.; Knight, B. W.; LePeq, F.; Wudl, F.; Yao, J.; Wilkins, C. L. *J. Org. Chem.* **1995**, *60*, 532.

65. Vasella, A.; Uhlmann, P.; Waldraff, C. A. A.; Diederich, F.; Thilgen, C. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1388.

66. (a) Bullard-Dillard, R.; Creek, K. E.; Scrivens, W. A.; Tour, J. M. unpublished results; (b) personal communication with Professor J. M. Tour, University of South Carolina, Department of Chemistry and Biochemistry.

67. Wilson, S. R.; Chin, E. *Fullerene Sci. Technol.* **1996**, *4*, 43.

68. Hamano, T.; Mashino, T.; Nagano, T.; Arakana, K.; Ryu, R.; Mashiko, S.; Hirobe, M. Presented at the Pacifichem-96 Meeting, Honolulu, Hawaii, December 1995; paper 1028.

69. Nelson, M. A.; Domann, F. E.; Bowden, G. T.; Hooser, S. B.; Fernando, Q.; Carter, D. E. *Toxicol. Ind. Health* **1993**, *9*, 623.

70. Zakharenko, L. P.; Zakharov, I. K.; Lunegov, S. N.; Nikiforov, A. A. *Dokl. Akad. Nauk.* **1994**, *335*, 261.

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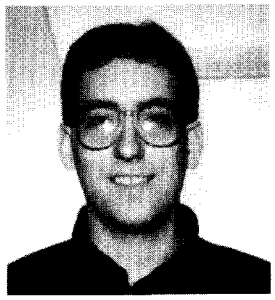
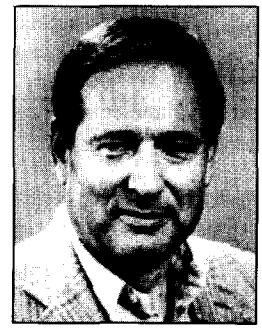
Biographical Sketch



Anton W. Jensen is a postdoctoral fellow in the laboratories of Professor David I. Schuster at New York University. He was born in Madison, Wisconsin, on 26 July 1966. He attended primary and secondary schools in Moscow, Idaho, home of the University of Idaho, where his father is a Professor of Spanish Literature. He attended the University of Idaho for 1 year (1984–1985) before leaving for Santiago, Chile to serve a 2 year mission for The Church of Jesus Christ of Latter-Day Saints. Subsequently, he attended Brigham Young University in Provo, Utah, where he earned his B.A. in Chemistry in 1990 and his Ph.D. in 1995 under the direction of Professor Steven A. Fleming. He is primarily interested in reaction mechanisms and is currently studying photcycloadditions to fullerenes in Professor Schusters photochemistry group. Starting in the Fall of 1996, he will be an Assistant Professor of Organic Chemistry at Central Michigan University.

Dr Stephen R. Wilson grew up in Houston, Texas, and attended Rice University, where he received his B.A. degree in Chemistry in 1969 and his M.A. and Ph.D. in 1972 working with Professor R. Turner. His thesis research involved development of the first synthesis of the skeleton of a novel antibiotic, marasmic acid. He then accepted a NIH postdoctoral fellowship at Caltech with Robert E. Ireland and began work on the total synthesis of the complex pentacyclic triterpene β-amyrin. In 1974, Dr Wilson joined the faculty at Indiana University and advanced to the rank of Associate Professor. In 1980 he moved his research group to New York University where he is currently Professor of Chemistry. His research group has carried out work on a wide range of topics, including the isolation and synthesis of natural products, development of new synthetic methods, computer-assisted drug design, electrospray mass spectrometry, and most recently fullerene chemistry.



David I. Schuster has been at New York University since 1961, where he is Professor of Chemistry. A native New Yorker, born on 13 August 1935, he received his B.A. at Columbia in 1956, summa cum laude, and his Ph.D. in 1961 from Caltech, where he worked on free radical chemistry of small-ring compounds with John D. Roberts. He began working in the field of mechanistic organic photochemistry during a postdoctoral year with Howard Zimmerman at Wisconsin in 1960–1961, and has continued to do research in this field at NYU. He is particularly known for his many contributions in the areas of enone and dienone photochemistry. He spent a year (1968–1969) learning techniques of flash photolysis in George Porter's laboratory at the Royal Institution in London, where he was involved in the first application of nanosecond flash photolysis to studies of triplet excited states. In recent years, laser flash photolysis and photoacoustic calorimetry have played an increasingly important role in his photochemical investigations, which have focused for the last 3 years on photochemical reactions involving fullerenes. Since 1975, he has been engaged in research in receptor biochemistry, involving pharmacological studies of drug–receptor interactions, photoaffinity labeling of receptors, purification of receptors by affinity chromatography, and characterization of cloned receptor proteins.