Synthesis and Molecular Modeling Studies of Fullerene−5,6,7-Trimethoxyindole−Oligonucleotide Conjugates as Possible Probes for Study of Photochemical Reactions in DNA Triple Helices

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The synthesis of novel functionalized fullerene derivatives containing DNA minor groove binders is reported. In order to construct sequence-specific probes for DNA photomodification, the compounds were attached to a triple helix forming oligonucleotide, and the formation of triplexes was monitored. The triplex formation was demonstrated, but the presence of fullerene moieties gives rise to a high degree of instability. Molecular modeling studies on these supramolecular structures provided useful information for the current study and for future developments.

Introduction

In the last few years, organofunctionalized derivatives of C_{60} fullerene have been intensively investigated for biological applications. This class of compounds exhibits a large variety of biological properties, such as HIV protease inhibition, neuroprotection, and induction of apoptosis.[1−3]

A most interesting feature of C_{60} is found in its photoinduced behavior. After irradiation, C_{60} is excited to a short-lived singlet state, which then converts into a long-lived triplet state.[4,5] This latter state may transfer energy to molecular oxygen with a very high quantum yield, generating singlet oxygen, which is responsible for a variety of biological effects. In particular, one very important aspect is represented by the ability of these compounds to induce DNA photocleavage.[6] Since fullerene activation can commonly be achieved by visible light, easily absorbed by blood and tissues, C_{60} can be used for in vitro experiments or for surface photochemical treatment, while the use of fullerene as a hydrophobic transporter through cellular membranes remains of great interest.

In most cases, DNA damage occurs at guanine bases, without significant specificity between the various G sites.[7,8] Some conjugates between C_{60} and nucleic acid specific agents (acridine,[9] netropsin[10]) have been reported, but no improvement in terms of selectivity has been obtained. It has been shown that only oligonucleotide−fullerene conjugates[11,12] are able to form relatively stable duplexes and triplexes with the complementary DNA sequences and to modify guanines in close proximity to fullerene locations photochemically.[11−13] Further development of this research might target improvement of the photocleavage efficiency of the conjugates. One of the possible ways is to stabilize their duplexes and triplexes and to increase their sequence selectivity. Dervan and co-workers, for example, have succeeded in dramatically stabilizing a triple helix and in increasing the recognition sequence by covalently binding a third strand to a sequence-specific minor groove binder.[14]

Our group has reported, in preliminary form, the synthesis of the first fullerene−trimethoxyindole−oligonucleotide conjugate **1** as a new tool for targeting the fullerene moiety to a desired DNA sequence.[15] The design strategy behind this derivative was based on a possible synergistic effect between a trimethoxyindole (TMI) unit and an oligonucleotide. In fact, TMI is a minor groove binder, characteristic of a class of natural alkylating antibiotics named duocarmycins, possessing high antitumor activity and high affinity and selectivity for DNA minor groove AT-rich sequences.[16] The oligonucleotide moiety was capable of inducing either duplex or triple helix formation, to provide high sequence selectivity, and of increasing water solubility, a major problem associated with C_{60} derivatives. In particular, the interaction of TMI with the minor groove could stabilize the triple helix. However, one of the problems was to find optimal lengths of linkers within oligonucleotide, minor groove binder, and fullerene in order

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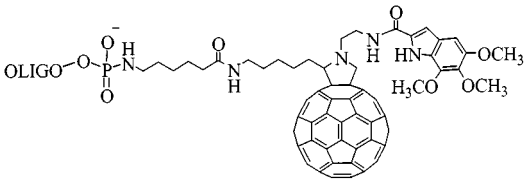
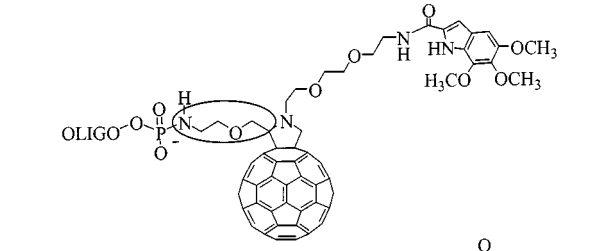
to ensure the correct positioning of each component in the complex.



1

OLIGO = 5'-TTT-TC*T-TTT-C*C*C*-C*C*C*-T-3'
C* = Methylated Cytosine

Here we report the synthesis and the first evaluation of a series of conjugates with spacers of varying lengths between C_{60} and oligonucleotide and TMI (**2**–**4**). (Figure 1). All these aspects were further explored by molecular modeling simulations, with the aim of optimizing the structural requirements indispensable for the DNA interaction and hence triple helix stabilization.



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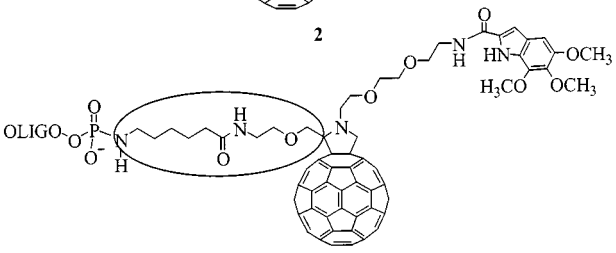

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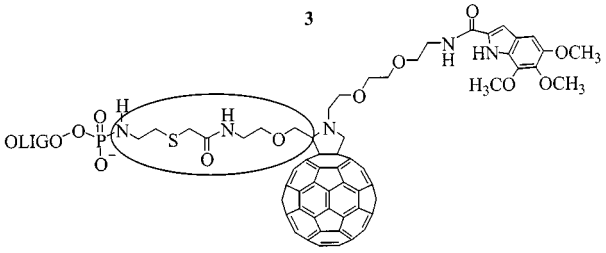

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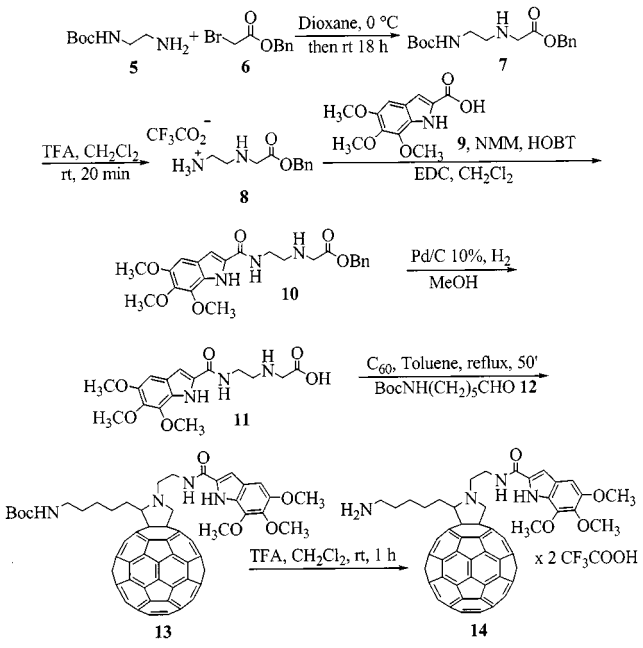
OLIGO = 5'-TTT-TC*T-TTT-C*C*C*-C*C*C*-T-3'
C*= Methylated Cytosine

Figure 1. Structures of the fullerene derivatives

Results and Discussion

Compounds **1**–**4** were prepared by the general synthetic strategy summarized in Schemes 1–5, according to the known procedure for the synthesis of fulleropyrrolidines, based on the 1,3-dipolar cycloaddition of azomethine ylides to C_{60}.[17,18] For the preparation of compound **1**, the appropriate amino acid was prepared by alkylation of the monoprotected (Boc) ethylenediamine **5** with benzyl α-bromoacetate (**6**) to afford the intermediate **7** (Scheme 1). After selective deprotection by TFA, amine **8** was obtained, and this was coupled to trimethoxyindole-2-carboxylic acid **9**[19] to afford amino ester **10**.



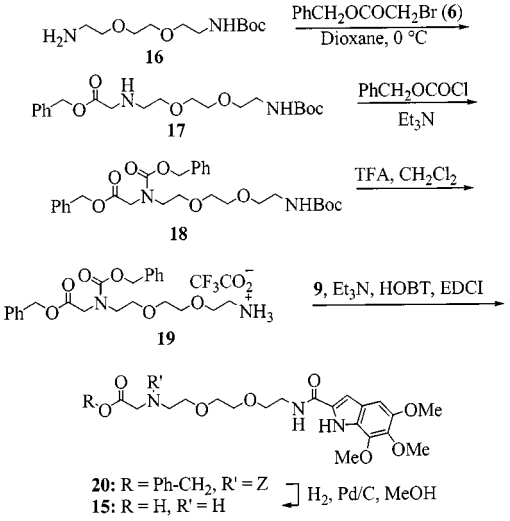
Scheme 1

This was deprotected at the carboxylic function by catalytic hydrogenation and the resulting acid **11** was allowed to react with C_{60} and *N*-Boc-protected 6-aminohexanal **12** to yield the desired multifunctional fulleropyrrolidine **13**. This compound was in turn deprotected by treatment with TFA to give the desired salt **14**, which was used for coupling reactions with the oligonucleotide.

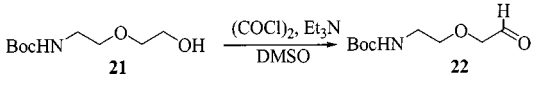
For compounds **2**–**4**, bearing oxygenated chains on the pyrrolidine ring, a similar approach was utilized, but the aldehyde and amino acid counterparts necessary for azomethine ylide generation were prepared as summarized in Schemes 2–3.

Amino acid counterpart **15** was prepared similarly to compound **11** (Scheme 1) with some modifications due to synthetic problems. Selective monoprotection of the 2,2′-(ethylenedioxy)diethylamine provided monoamino derivative **16**, which was in turn alkylated with **6** to give **17** in good yield. In this case it was necessary to protect the glycine nitrogen atom of **17** as the benzyloxycarbonyl derivative **18** to avoid secondary reactions and lower yields (Scheme 2).

Subsequent *N*-Boc deprotection by TFA treatment (**19**) and coupling with 5,6,7-trimethoxyindole-2-carboxylic acid (**9**)[19] gave conjugate **20**, which, after deprotection at the carboxylic function by catalytic hydrogenation, afforded the corresponding acid **15**. When starting from *N*-Boc-protected 2-(2-aminoethoxy)ethanol **21**,[20] by oxidation under Swern conditions, the appropriate aldehyde **22** was prepared and used immediately without purification, because of its high instability (Scheme 3).

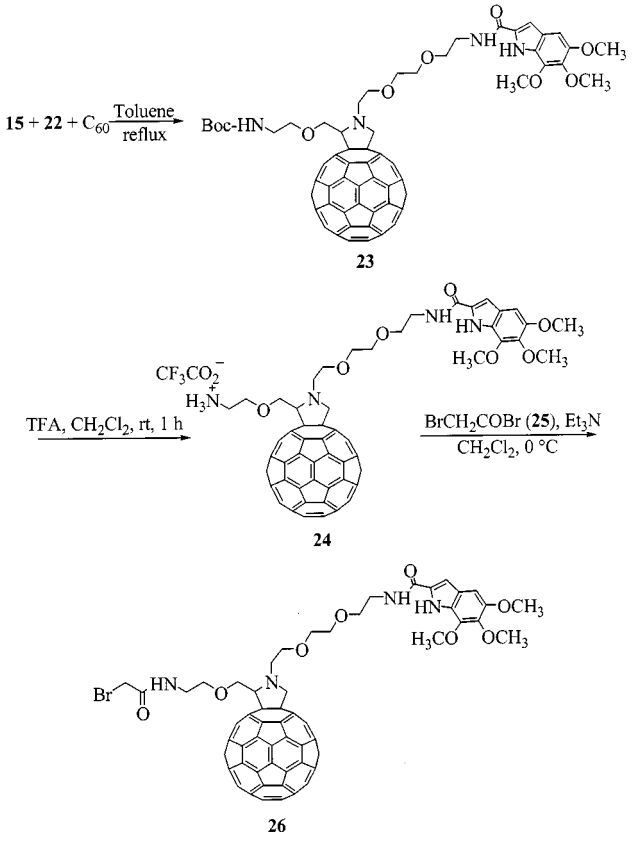


Scheme 2



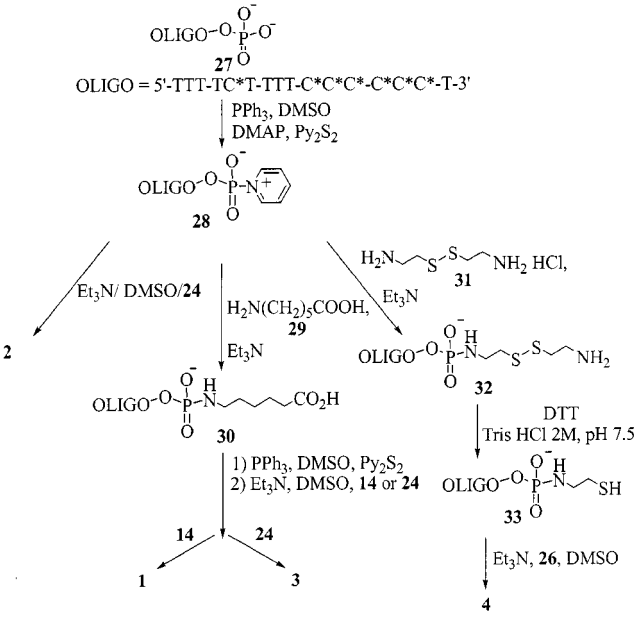
Scheme 3

The desired bis(functionalized) fulleropyrrolidine **23** was obtained by treatment of C_{60}, amino acid **15**, and aldehyde **22** in refluxing toluene (Scheme 4). Derivative **23** was in turn deprotected at the amine function by treatment with TFA to produce **24**, which was treated with α-bromoacetyl bromide (**25**) at 0 °C to obtain derivative **26**.



Scheme 4

The conjugates between fullerene derivatives **14**, **24**, and **26** and the 5′-phosphorylated oligonucleotide pTTTTC* TTTTC*C*C*C*C*C*T (where C* is 5-methylcytosine, to facilitate the protonation of the cytidine moiety necessary for triplex formation) were synthesized by a previously reported general synthetic strategy,[21,22] summarized in Scheme 5. The terminal phosphate oligonucleotide (16-mer) **27** was activated (**28**) by means of Mukaiyama reagents:[23,24] triphenylphosphane−dipyridyl 2,2′-disulfide in the presence of 4-(dimethylamino)pyridine, precipitated by 10 vol-equiv. of lithium perchlorate in acetone and attached to the appropriate amino-containing spacer utilized for conjugate formation in organic or water/organic solution as described by Grimm et al.[24]



Scheme 5

Different conjugate synthesis methods are indicated in Scheme 5. When **28** was treated with fulleropyrrolidine **24** in the presence of triethylamine in dimethyl sulfoxide, the oligonucleotide sequence was directly linked to the amino group of the branched chain of the C_{60} derivative through its terminal phosphate (**2**). However, the yield of the direct reaction was relatively low (less than 30%).

Alternatively, **28** could be coupled to the amino group of 6-aminocaproic acid (**29**) to yield derivative **30**, which, after treatment with **14** or **24** under Mukaiyama conditions, afforded the desired derivatives **1** or **3**, respectively. In this case, better yields of conjugate (about 80%) were obtained and a longer linker between the fullerene moiety and the oligonucleotide sequence was introduced.

A quite different approach was used with cysteamine as a spacer. In this case, the activated oligonucleotide was treated with cystamine **31**. After reprecipitation by ethanol/sodium acetate, the disulfide bond of **32** was reduced with

dithiothreitol (DTT) to yield **33**, which was coupled with alkylating derivative **26** in the presence of triethylamine to afford derivative **4**.

Purification of **1**−**4** by HPLC was not possible, due to adsorption of the derivatives onto the stationary phase (both reverse phase and ion-exchange resins). Purification was therefore achieved by electrophoresis in 1% agarose gel in tris(acetate) buffer with 0.1% of triton X-100 in the samples. The electrophoresis revealed one homogeneous, but quite disperse, colored band migrating more slowly than the unmodified oligonucleotide. The product was isolated by excision of the colored band and digestion of agarose with β-agarase.

The interest of conjugates between oligonucleotides and fullerenes lies in the possibility of directing fullerene derivatives to sequence-specific sites on the target RNA or DNA. As a model, we used a triple helix forming polypurine region of HIV proviral DNA. It is known that HIV genes *pol* and *nef* contain the same 16-*mer* polypurine,[25,26] capable of forming a triple helix with the corresponding polypyrimidine oligonucleotide. The TMI residue might stabilize the conjugate−DNA interaction by minor groove binding.

Preliminary gel retardation experiments, performed with excesses of the triplex-forming oligonucleotide, demonstrated that derivative **1** readily forms a triple helix with a purine−pyrimidine-rich 29-mer fragment of HIV proviral DNA (Figure 2, panel A; for sequence see Exp. Sect.), as well as other derivatives (**2**−**4**, Figure 2, panel B). In these cases, however, the triplex is less stable and the triplex formation is not complete.

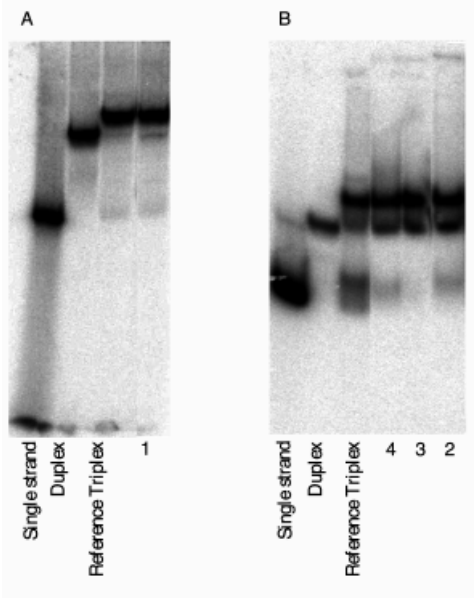


Figure 2. Panel A: nondenaturating gel electrophoresis of triplex formation by derivative **1**; panel B: nondenaturating gel electrophoresis of triplex formation by **2**−**4**

Despite the evidence of triplex formation in gel retardation experiments, no detectable transition for fullerene-modified triplexes was observed in triplex thermal denaturation experiments, as shown in Figure 3. For a nonmodified third strand, a clear "triplex-duplex" transition was observed at 25 °C.

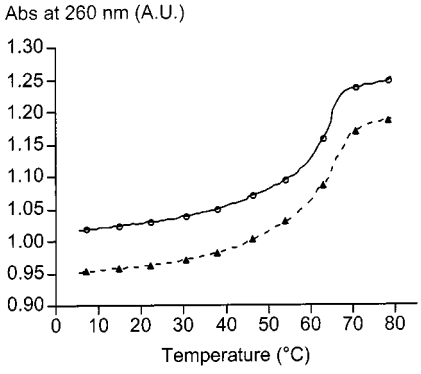


Figure 3. Melting curves of compound **3** (triangles) and compound **4** (circles); the behavior of the other compounds does not differ from those reported in this figure

Measurement of dissociation constants of triplexes by gel-retardation experiments indicated that this constant is 50 times higher ($2 \cdot 10^{-5}$ M compared to $4.3 \cdot 10^{-7}$ M for the unmodified third strand).

All the reported data show that the presence of a hydrophobic fullerene moiety acts against stabilization, with a decrease in the time of residence of the third strand in the duplex. As a consequence, we did not observe a clear, triplex-directed, sequence-specific cleavage of target duplex with the fullerene-containing conjugate after irradiation of the triple helix with short-wave visible light (> 300 nm).

A preliminary molecular modeling study was carried out, to better analyze the behavior of conjugates **1** and **3**. In particular, we exhaustively explored the conformational space of the bis(functionalized) fulleropyrrolidine moiety around the oligonucleotide structure, by using the Stochastic Conformational Search (SCS) approach (see Computational Methodologies Section). The lowest-energy conformers of both **1** and **3** derivatives obtained after SCS are shown in Figure 4.

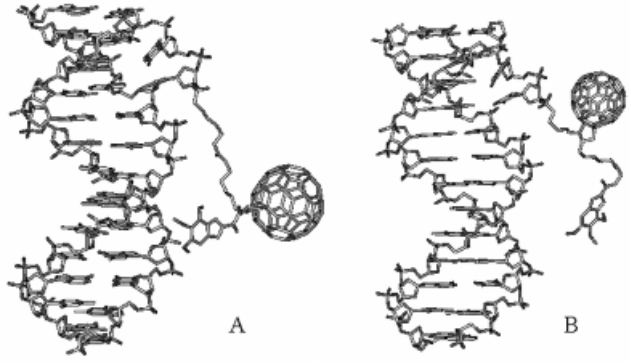


Figure 4. Partial stereoview of the most stable conformer of the fullerene−trimethoxyindole−oligonucleotide conjugates **1** (panel A) and **3** (panel B) viewed perpendicular to the principal axes of DNA (see Exp. Sect. for details); hydrogen atoms and most of the triple helix forming oligonucleotide are omitted for clarity

In both examples, fulleropyrrolidine and TMI moieties were found to be far away from DNA major and minor grooves in aqueous environments. From a theoretical point of view, one critical determinant might be appropriate separation between the triple helix forming oligonucleotide and the fulleropyrrolidine. In fact, if the third strand of the triplex is localized in the DNA major groove, the linker bearing the minor groove binders must be long enough to encircle the DNA strand from the major to the minor groove to direct a TMI moiety into the target. In contrast, it was evident from the computational analysis that the C_{60} spheroid was in both cases at some distance from the DNA, due to a longer spacer between fullerene and oligonucleotide chain, which also avoided the intercalation of TMI with the minor groove. In addition, strong steric and electrostatic repulsions between the negatively charged phosphate groups and the fulleropyrrolidine moiety played a crucial role in determining incorrect positioning of fullerene in the ternary oligonucleotide−fullerene−minor groove binder conjugate. On the other hand, the size of the fullerene does not allow it to enter the minor groove. For this reason, the C_{60} moiety has to be positioned close to the oligonucleotide in order to permit its entry into the major groove (6 Å). During the stochastic conformational analysis we found very few unstable conformations in which the fullerene was located in the major groove. In view of the energy balance of the ternary oligonucleotide−fullerene−TMI conjugate formation, the presence of the fullerene seems to be undesirable not only in the water phase, due to its high hydrophobicity, but also, taking account of the peculiar hydrophilic properties of that environment, in the DNA major groove. From this study, it is evident that the distances between oligonucleotide, C_{60}, and TMI seem to play a fundamental role in DNA recognition and interaction, as well as in the charge located on the fullerene moiety.

Conclusion

In conclusion, a series of C_{60}−oligonucleotide−TMI conjugates has been prepared. Molecular modeling support has demonstrated that the problem of optimal spacing between oligonucleotide, fullerene, and minor groove binder seems to be critical. New derivatives with elongated linkers between fullerene and TMI units, which might permit the fullerene moiety to reside in a major groove and which would be long enough to circle the DNA strand are currently under development in our laboratories, as are other, more specific, minor groove binders, and the separate positioning of fullerene and MGB from different termini.

Experimental Section

General Remarks: FT-IR spectra were recorded with a Jasco FT/IR-200 spectrophotometer with NaCl cells or compound/KBr mixtures (DRIFT-IR system). ^{1}H and ^{13}C spectra were recorded with a Varian Gemini 200 spectrometer (^{1}H at 200 MHz and ^{13}C at 50 MHz) in $CDCl_3$, unless otherwise noted. Chemical shifts are given in parts per million (δ) relative to tetramethylsilane. ES-MS spectra were obtained with a PE SCIEX API 1 spectrometer (THF/methanol, 4:1, unless otherwise noted), the EI-MS spectra with a 7070H VG Micromass. UV/Vis absorption spectra were recorded with a Perkin−Elmer Lambda 20 UV/Vis or Kontron 923 spectrophotometer. Yields are reported as absolute values without taking C_{60} recovery into account. C_{60} was purchased from Bucky-USA (99.5%), all other reagents and solvents were used as purchased from Fluka, Aldrich, J. T. Baker, and Cambridge Isotope Laboratories; silica gel NM Kieselgel 60 (70−230 mesh ASTM) was obtained from Macherey−Nagel and Merck and the oligonucleotide from Eurogentec. Trimethoxyindole-2-carboxylic acid (**9**),[19] aldehyde **12**,[27] and monoprotected diamines **5**[28] and **16**[29] were prepared according to reported procedures. It is known that most fullerene derivatives do not burn properly, and experimental elemental analyses therefore often deviate from the calculated values.[30] DCM = dichloromethane.

Synthesis of Fulleropyrrolidine 13: A solution of **6** (2.30 g, 10.0 mmol) in 1,4-dioxane (20 mL) was added dropwise at 0 °C over 1 h to a solution of **5** (5.00 g, 31.2 mmol) in 1,4-dioxane (30 mL), and the reaction mixture was stirred overnight. The solvent was evaporated under reduced pressure, and the residue was dissolved in water (70 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were dried with anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude residue was purified by chromatography (ethyl acetate/petroleum ether, 1:1, followed by pure ethyl acetate) to afford the desired compound **7** as an oil (2.16 g, 7.0 mmol, yield 70%). FT-IR: $\tilde{\nu}$ = 3347, 2974, 2930, 1738, 1694, 1517, 1170, 968, 751 cm^{-1}. ^{1}H NMR: δ = 1.44 (s, 9 H), 2.74 (t, J = 5.9 Hz, 2 H), 3.19 (m, 2 H), 3.45 (s, 2 H), 4.98 (br. s, 1 H), 5.17 (s, 2 H), 7.36 (s, 5 H). ^{13}C NMR: δ = 28.3, 49.7, 50.4, 66.6, 68.0, 79.1, 128.3, 128.3, 128.5, 135.4, 156.0, 172.3. EI-MS: m/z (%) = 309 (30) [M + 1]$^+$, 253 (8), 91 (100). Trifluoroacetic acid (10 mL) was added to a solution of **7** (1.33 g, 4.3 mmol) in DCM (10 mL), and the reaction mixture was stirred for 20 min at room temp. The solvent was evaporated and the salt was washed with toluene and dried under vacuum to afford an oil (**8**) (1.38 g, 4.3 mmol, yield 99%). ES-MS: m/z = 209 [MH$^+$]. 4-Methylmorpholine (220 μL, 2.0 mmol) was added to a solution of **8** (0.32 g, 1.0 mmol) in DCM (20 mL). In a separate flask, a solution of **9** (0.25 g, 1.0 mmol), HOBT (0.26 g, 2.0 mmol), and EDC·HCl (0.38 g, 2.0 mmol) in DCM (10 mL) was stirred for 15 min and then added dropwise to the solution of **8** at 0 °C. After 15 min, the crude material was washed with water (20 mL) and extracted with DCM (3 × 10 mL), the organic phases were dried with $CaCl_2$, and the solvent was evaporated under reduced pressure. The product **10** was purified by flash chromatography (ethyl acetate) (0.13 g, 0.3 mmol, yield 29%). DRIFT-IR: $\tilde{\nu}$ = 3315, 2930, 1735, 1635 cm^{-1}. ^{1}H NMR: δ = 2.88 (t, J = 5.7 Hz, 2 H), 3.50 (s, 2 H), 3.52 (m, 2 H), 3.86 (s, 3 H), 3.90 (s, 3 H), 4.03 (s, 3 H), 5.17 (s, 2 H), 6.76 (s, 1 H), 6.80 (s, 1 H), 7.04 (bt, 1 H), 7.34 (s, 5 H), 9.23 (br. s, 1 H). ^{13}C NMR: δ = 48.5, 50.4, 56.4, 68.2, 123.4, 125.6, 128.6, 128.7, 130.9, 132.5, 135.4, 139.0, 139.8, 139.9, 149.9, 167.8, 172.6. A solution of **10** (0.13 g, 0.3 mmol) in methanol (20 mL) was stirred for 1 h under hydrogen pressure in the presence of Pd/C (0.05 g). Compound **11** was isolated by filtration through Celite and the solvent was evaporated under reduced pressure (0.10 g, 0.3 mmol, yield 99%). ^{1}H NMR: δ = 3.00 (m, 2 H), 3.47−3.75 (m, 4 H), 3.48 (s, 3 H), 3.66 (s, 3 H), 3.73 (s, 3 H), 6.33 (s, 1 H), 6.83 (s, 2 H), 8.49 (br. s, 1 H), 10.71 (br. s, 1 H). ^{13}C NMR: δ = 46.8, 47.6, 55.8, 61.3, 123.3, 126.1, 128.8, 130.1, 130.9, 132.4, 138.8, 139.8, 162.9, 172.2. ES-MS: m/z = 352 [MH$^+$]. A mixture of C_{60} (0.24 g, 0.3 mmol), amino acid **11** (0.12 g, 0.3 mmol), and aldehyde

12 (0.22 g, 1.0 mmol) in dry toluene (300 mL) was heated to reflux for 50 min. After evaporation of the solvent, the crude residue was purified by flash chromatography (toluene followed by toluene/2-propanol, 9:1). The solvent was evaporated, and compound **13** was then dissolved in DCM and precipitated by addition of methanol (0.08 g, 0.1 mmol, yield 19 %). DRIFT-IR: $\tilde{\nu}$ = 3330, 2935, 2850, 1690, 1645, 1245, 1169, 526 cm^{-1}. ^1H NMR: δ = 1.43 (s, 9 H), 1.49 (m, 2 H), 1.89 (m, 2 H), 2.45 (m, 2 H), 3.11 (m, 2 H), 3.25 (m, 1 H), 3.85 (s, 3 H), 3.89 (s, 3 H), 3.95−4.08 (m, 4 H), 4.05 (s, 3 H), 4.30 (d, *J* = 10.5 Hz, 1 H), 4.37 (t, *J* = 5.9 Hz, 1 H), 4.52 (m, 1 H), 4.98 (d, *J* = 10.3 Hz, 1 H), 6.75 (s, 1 H), 6.93 (s, 1 H), 7.30 (br. s, 1 H), 9.27 (br. s, 1 H). ^{13}C NMR: δ = 27.0, 28.5, 29.9, 31.0, 43.6, 56.3, 56.8, 60.4, 61.1, 71.2, 79.3, 123.3, 125.7, 130.6, 131.1, 135.4, 135.6, 135.8, 136.7, 139.7, 140.2, 141.7, 141.9, 142.0, 142.1, 142.2, 142.6, 143.0, 144.3, 144.4, 144.6, 145.1, 145.2, 145.5, 145.9, 146.0, 146.1, 146.2, 146.3, 147.1, 150.0, 153.2, 154.6, 156.1, 166.6. ES-MS: *m*/*z* = 1225 [MH^+]. UV/Vis (cyclohexane): λ_{max} = 254, 312, 431, 703 nm. − $C_{86}H_{40}N_4O_6$ (1225.30): calcd. C 84.30, H 3.29, N 4.57; found C 82.20, H 3.92, N 4.30.

Synthesis of Salt 14: Trifluoroacetic acid (10 mL) was added to a solution of **13** (0.07 g, $5.7 \cdot 10^{-5}$ mol) in DCM (10 mL), and the reaction mixture was stirred for 10 min at room temperature. The solvent was evaporated, and the salt was washed with toluene and dried under vacuum (0.07 g, $5.2 \cdot 10^{-5}$ mol, yield 91%). ES-MS: *m*/*z* = 1126 [M^+]. UV/Vis (THF): λ_{max} = 272, 327, 431 nm. $C_{85}F_6H_{34}N_4O_8$ (1353.23): calcd. C 75.44, H 2.53, N 4.14; found C 71.4, H 2.69, N 4.17.

Synthesis of Ester 17: A solution of **6** (1.5 mL, 9.5 mmol) in 1,4-dioxane (50 mL) was added dropwise to a solution of **16** (7.1 g, 28.6 mmol) in 1,4-dioxane (100 mL) and the mixture was stirred overnight at room temperature. The solvent was evaporated and the residue was dissolved in ethyl acetate (30 mL) and washed with water (3 × 10 mL). The organic phase was dried with Na_2SO_4 and filtered, and the solvents were evaporated. The crude material was then purified by flash chromatography (eluent ethyl acetate/petroleum ether, 1:1, followed by ethyl acetate), yielding a colorless oil (3.2 g, 8.0 mmol, yield 84%). FT-IR: $\tilde{\nu}$ = 3347, 2871, 1712, 1512, 1458, 1368, 1248, 1175, 966, 865, 747, 701 cm^{-1}. ^1H NMR: δ = 1.41 (s, 9 H), 2.78 (t, *J* = 5.2 Hz, 2 H), 3.28 (m, 2 H), 3.46−3.51 (m, 4 H), 3.51−3.59 (m, 6 H), 5.15 (s, 2 H), 5.21 (br. s, 1 H), 7.32 (m, 5 H). ^{13}C NMR: δ = 28.5, 40.3, 40.4, 48.6, 50.8, 66.5, 70.1, 70.2, 70.6, 79.0, 128.2, 128.5, 135.5, 155.9, 172.1. EI-MS: *m*/*z* (%) = 397 (20) [M + 1]$^+$, 205 (100), 161 (40), 91 (55).

Synthesis of 18: ZCl (5.1 mL, 35.7 mmol) was added to a solution of **17** (6.0 g, 15.1 mmol) and TEA (2.7 mL, 19.7 mmol) in dried DCM (50 mL); the mixture was stirred over a period of 20 min, then washed with acidic, basic, and neutral water. The organic phase was dried with $CaCl_2$ and filtered, and the solvents were evaporated under reduced pressure. The crude material was purified by flash chromatography (eluent petroleum ether/ethyl acetate, 7:3, followed by 6:4), to obtain **18** as an oil (6.8 g, 12.8 mmol, yield 85%). FT-IR: $\tilde{\nu}$ = 3367, 2936, 1707, 1508, 1460, 1244, 1178, 999, 741 cm^{-1}. ^1H NMR: δ = 1.41 (s, 9 H), 3.25 (q, *J* = 5.1 Hz, 2 H), 3.34−3.70 (m, 10 H), 4.15 and 4.19 (s, 2 H), 4.99 (br. s, 1 H), 5.07 and 5.08 (the peaks are split because of the slow rotation around the urethane or amide C−N bonds, s, 2 H), 5.15 (s, 2 H), 7.24−7.41 (m, 10 H). ^{13}C NMR: δ = 28.3, 47.8, 48.4, 50.1, 66.6, 66.7, 67.3, 67.4, 69.9, 70.1, 70.2, 79.0, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 135.3, 136.2, 155.7, 169.6. MS-ES: *m*/*z* = 531 [M + 1]$^+$.

Synthesis of Salt 19: TFA (10 mL) was added to a solution of **18** (3.0 g, 5.7 mmol) in 10 mL of DCM, and the mixture was stirred over a period of 1 h. The solvent and TFA were evaporated and the residue was washed with toluene (3.2 g, 5.7 mmol, yield 99%). FT-IR: $\tilde{\nu}$ = 2944, 1690, 1463, 1360, 1196, 997, 702 cm^{-1}. ^1H NMR: δ = 2.90−3.20 (m, 2 H), 3.31−3.71 (m, 10 H), 4.07 and 4.13 (s, 2 H), 5.05 and 5.10 (s, 2 H), 5.14 (s, 2 H), 7.10−7.40 (m, 10 H). ^{13}C NMR: δ = 39.7, 48.1, 48.6, 49.9, 50.5, 66.6, 67.1, 67.7, 69.4, 70.1, 70.2, 127.6, 128.1, 128.2, 128.4, 128.5, 128.6, 135.3, 136.2, 156.4, 161.1, 161.9, 169.8. MS-ES: *m*/*z* = 431 [M^+].

Synthesis of Amino Ester 20: TEA (776 μL, 5.6 mmol) was added to a solution of **19** (1.5 g, 2.7 mmol) in 10 mL of DCM. In a separate flask, a solution of **9** (0.70 g, 2.8 mmol), EDC·HCl (0.76 g, 5.6 mmol), and HOBT (0.79 g, 5.6 mmol) in 10 mL of DCM was stirred for 15 min and then added dropwise to the solution of **19** at 0 °C. After 30 min, the solution was washed with water (3 × 10 mL) and dried with $CaCl_2$, and the solvent was evaporated under reduced pressure. The crude material was purified by flash chromatography (ethyl acetate) to afford the desired compound as an oil (0.99 g, 1.5 mmol, yield 56%). FT-IR: $\tilde{\nu}$ = 3633, 3268, 2816, 1728, 1633, 1564, 1409, 1267, 1118, 704, 458 cm^{-1}. ^1H NMR: δ = 3.45−3.70 (m, 14 H), 3.85 (s, 3 H), 3.89 (s, 3 H), 4.00 (s, 3 H), 4.16 and 4.21 (s, 2 H), 5.05 and 5.09 (s, 2 H), 5.14 (s, 2 H), 6.77 (d, *J* = 2.2 Hz, 1 H), 6.84 and 6.87 (d, *J* = 2.2 Hz, 1 H), 6.93 and 7.01 (br. s, 1 H), 7.16−7.47 (m, 10 H), 9.33 and 9.39 (br. s, 1 H). ^{13}C NMR: δ = 39.5, 48.1, 48.6, 50.4, 50.6, 56.3, 61.2, 61.5, 67.0, 67.6, 67.7, 70.2, 70.3, 70.4, 103.0, 103.3, 123.4, 125.7, 127.8, 127.9, 128.1, 128.2, 128.4, 128.5, 128.6, 135.3, 136.3, 139.0, 139.9, 149.8, 156.0, 156.3, 161.4, 170.1. EI-MS: *m*/*z* (%) = 663 (15) [M^+], 233 (80) [M^+ − CO-TMI], 91 (100).

Synthesis of Amino Acid 15: A solution of **20** (0.61 g, 0.9 mmol) in 10 mL of methanol was stirred for 2 h under hydrogen pressure, with 10% Pd/C (0.05 g) as catalyst. The crude material was purified by filtration through Celite; the solvent was then evaporated under reduced pressure, yielding a solid after precipitation with diethyl ether (0.40 g, 0.9 mmol, yield 99%), m.p. 115−116 °C. DRIFT-IR: $\tilde{\nu}$ = 3264, 1627, 1405, 1264, 1105 cm^{-1}. ^1H NMR: δ = 2.90−4.19 (m, 14 H), 3.77 (s, 3 H), 3.83 (s, 3 H), 3.94 (s, 3 H), 6.69 (s, 1 H), 7.11 (s, 1 H), 8.31 (br. s, 1 H), 10.55 (br. s, 1 H). ^{13}C NMR: δ = 23.1, 23.9, 29.1, 56.3, 61.3, 61.5, 69.9, 123.4, 126.0, 131.2, 139.0, 139.8, 149.6, 162.1, 196.0. ES-MS: *m*/*z* = 440 [MH^+].

Synthesis of Fulleropyrrolidine 23: A solution of DMSO (1.7 mL, 24.4 mmol) in DCM (10 mL) was added dropwise over a period of 1 h to a solution of oxalyl chloride (950 μL, 11.2 mmol) in 10 mL of DCM at −60 °C under N_2. The reaction mixture was stirred for 30 min, and a solution of **21**[20] (1.00 g, 4.9 mmol) in 10 mL of DCM was then added dropwise over 30 min and the mixture was stirred for 30 min. To this mixture, a solution of TEA (43.9 mmol, 6.15 mL) in 10 mL of DCM was added dropwise over 30 min. The whole mixture was allowed to react overnight. The solvent was then evaporated and the crude material was diluted with ethyl acetate and washed with acidic, basic, and neutral water. The organic phase was dried and concentrated under vacuum, yielding **22** as an oil. The compound was used without any further purification because of its instability. It decomposed into a number of unidentified products in a matter of a few hours. EI-MS: *m*/*z* (%) = 203 (10) [M^+], 202 (20) [M − 1]$^+$, 186 (80), 149 (100), 130 (28). A mixture of C_{60} (0.46 g, 0.6 mmol), amino acid **15** (0.55 g, 1.2 mmol), and aldehyde **22** (0.64 g, 3.2 mmol) in toluene (500 mL) was heated to reflux for 50 min. After evaporation of the solvent, the crude material was purified by flash chromatography (toluene followed by toluene/2-propanol, 9:1). The solvent was evaporated and compound **23** was dissolved in DCM and precipitated by methanol (0.18 g, 0.1 mmol, yield 21%). DRIFT-IR: $\tilde{\nu}$ = 3439, 2922, 1707,

1644, 1257, 1106, 762, 526 cm^{-1}. ^1H NMR (400 MHz): δ = 1.39 (s, 9 H), 3.06−3.93 (m, 14 H), 3.84 (s, 3 H), 3.87 (s, 3 H), 3.93−4.13 (m, 2 H), 4.02 (s, 3 H), 4.22 (d, *J* = 9.9 Hz, 1 H), 4.26−4.38 (m, 2 H), 4.38−4.53 (m, 1 H), 4.95 (d, *J* = 9.5 Hz, 1 H), 5.01 (br. s, 1 H), 6.75 (s, 1 H), 6.84 (s, 1 H), 6.99 (br. s, 1 H), 9.25 (br. s, 1 H). ^{13}C NMR (100 MHz): δ = 28.05, 29.3, 39.1, 52.0, 56.0, 60.7, 61.1, 69.5, 69.7, 70.0, 70.2, 73.1, 76.9, 100.3, 102.6, 123.1, 125.4, 135.1, 135.6, 136.0, 138.7, 139.2, 139.5, 139.6, 139.9, 140.0, 141.5, 141.7, 141.8, 142.4, 142.8, 142.9, 144.1, 144.2, 144.5, 144.9, 145.0, 145.2, 145.5, 145.7, 145.9, 146.0, 146.2, 146.7, 147.0, 149.8, 153.6, 154.2, 155.4, 155.7, 161.0. UV/Vis (cyclohexane): λ_{max} = 258, 273, 331, 430, 703 nm. ES-MS: *m*/*z* = 1301 [MH^+]. $C_{88}H_{44}N_4O_9$ (1300.35): calcd. C 81.23, H 3.31, N 4.31; found C 78.09, H 3.27, N 4.26.

Synthesis of Salt 24: Trifluoroacetic acid (5 mL) was added to a solution of **23** (0.07 g, 53.6 μmol) in DCM (5 mL), and the reaction mixture was stirred for 10 min at room temperature. The solvent was evaporated, and the solid residue was washed with toluene and dried under vacuum (0.07 g, 49.3 μmol, yield 92%). DRIFT-IR: $\tilde{\nu}$ = 2873, 1680, 1459, 1194, 1129, 716, 520 cm^{-1}. ^1H NMR: δ = 2.96−4.32 (m, 16 H), 3.80 (s, 3 H), 3.83 (s, 3 H), 4.00 (s, 3 H), 4.32−4.68 (m, 3 H), 4.89 (br. s, 1 H), 5.26 (d, *J* = 10.9 Hz, 1 H), 6.74 (s, 1 H), 7.07 (s, 1 H), 7.95 (br. s, 1 H), 8.65 (br. s, 2 H). UV/Vis (DCM): λ_{max} = 272, 320, 430, 705 nm. ES-MS: *m*/*z* = 1201 [M^+]. $C_{87}H_{38}F_6N_4O_{11}$ (1429.28): calcd. C 73.11, H 2.68, N 3.92; found C 74.09, H 2.68, N 4.22.

Synthesis of 26: α-Bromoacetyl bromide (**25**) (6 μL, 68.0 μmol) was added at 0 °C to a solution of **24** (0.09 g, 68.0 μmol) and TEA (28.6 μL, 0.2 mmol) in 10 mL of DCM, and the mixture was stirred for 30 min. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography (toluene/2-propanol, 95:5). Compound **26** is a brown solid (0.03 g, 22.0 μmol, yield 32%). DRIFT-IR: $\tilde{\nu}$ = 3311, 3075, 2996, 1644, 1555, 1415, 1262, 1113, 833, 758, 478. ^1H NMR: 3.08−3.94 (m, 14 H), 3.85 (s, 3 H), 3.87 (s, 3 H), 3.89 (s, 2 H), 3.96−4.12 (m, 2 H), 4.02 (s, 3 H), 4.21 (d, *J* = 9.9 Hz, 1 H), 4.15−4.38 (m, 2 H), 4.42−4.67 (m, 2 H), 4.93 (d, *J* = 9.9 Hz, 1 H), 6.77 (s, 1 H), 6.83 (d, *J* = 2.2 Hz, 1 H), 6.96 (br. s, 1 H), 9.22 (br. s, 1 H). UV/Vis (DCM): λ_{max} = 274, 330, 430, 702 nm. ES-MS: *m*/*z* = 1324 [MH^+]. $C_{85}H_{37}BrN_4O_8$ (1322.17): calcd. C 77.22, H 2.82, N 4.24; found C 75.09, H 2.59, N 4.22.

Synthesis of Conjugate 30:[24] 5′-Phosphorylated commercial oligonucleotide ($3-5\cdot10^{-6}$ g, 0.06−0.10 μmol) was precipitated by successive addition of 8% CTAB. The dried CTAB salt was dissolved in DMSO (50 μL) and to this were added 4-(dimethylamino)-pyridine ($5.0\cdot10^{-3}$ g, 41 μmol) and 2,2′-dipyridyl disulfide ($6.6\cdot10^{-3}$ g, 30 μmol) in DMSO (25 μL), together with triphenylphosphane ($7.9\cdot10^{-3}$ g, 30.0 μmol) in DMSO (25 μL). After 20 min at room temperature, oligonucleotide **28** was precipitated with 2% $LiClO_4$ in acetone and dissolved in water. Aqueous 6-aminocaproic acid (**29**) (50 μL, $6.0\cdot10^{-3}$ g, 46.0 μmol) and TEA (9 μL) were immediately added to the solution of activated oligonucleotide. The reaction mixture was left for 2 h at room temperature. The oligonucleotide derivative **30** was then precipitated with $LiClO_4$/acetone and washed with acetone.

Synthesis of Conjugates 1 and 3: Oligonucleotide derivative **30** (0.06−0.10 μmol) was again precipitated from water solution with 8% CTAB, and the precipitate was dried under vacuum and redissolved in DMSO (50 μL). 2,2′-Dipyridyl disulfide ($3.3\cdot10^{-3}$ g, 15 μmol) in DMSO (25 μL) and triphenylphosphane ($3.9\cdot10^{-3}$ g, 15 μmol) in DMSO (20 μL) were added. After 20 min, a solution of fullerene derivative **14** or **24** (0.5 μmol) and TEA (5 μL) in DMSO (10 μL) was added. After 20 h at room temperature, the oligonucleotide was isolated by precipitation with NaOAc/EtOH, washed with EtOH, dried under vacuum, and redissolved in water.

Synthesis of Conjugate 2: Oligonucleotide derivative **28** (0.06−0.10 μmol) was redissolved in DMSO (50 μL) and a solution of fullerene derivative **24** (0.5 μmol) and TEA (9 μL) in DMSO (10 μL) was added. After 20 h at room temperature, the oligonucleotide was isolated by precipitation with NaOAc/EtOH, washed with EtOH, dried under vacuum, and redissolved in water.

Synthesis of Conjugate 33: After precipitation with 2% $LiClO_4$, oligonucleotide derivative **28** (0.06−0.1 μmol) was dissolved in 50 μL of H_2O, and TEA (9 μL) and cystamine hydrochloride **31** ($5\cdot10^{-3}$ g) were added to the solution. After 1 h at room temperature, the oligonucleotide was isolated by precipitation with NaOAc/EtOH, washed with EtOH, dried under vacuum, and redissolved in water (40μL). DTT ($1.7\cdot10^{-3}$ g) and Tris/HCl (2 M, pH = 7.5, 1 μL) were added. The reaction was carried out for 1 h and the oligonucleotide **33** was precipitated with degassed NaOAc/EtOH and then washed twice with degassed EtOH, dried under vacuum, and redissolved in degassed water (20 μL).

Synthesis of Conjugate 4: A DMSO solution (150 μL) of **26** ($5.0\cdot10^{-4}$ g) and TEA (9 μL) were added to a water solution of **33** (0.06−0.1 μmol in 20 μL). After 20 h, conjugate **4** was precipitated with NaOAc/EtOH, washed with EtOH, dried under vacuum, redissolved in water and reprecipitated.

Purification of Conjugates 1−4: Oligonucleotide−fullerene conjugates in 40 μL of 7 M urea/0.1% triton X-100 were loaded into a well in a 1% low melting temperature NuSieve GTG agarose (BioWhittaker Molecular Applications) slab in a standard tris(acetate) buffer. Electrophoresis at 150 V continued while the colored band was not too diffuse. In a control well, a sample of nonconjugated aminocaproic acid derivative of the oligonucleotide was added. The slab was analyzed by UV-shadowing techniques; migration of the control derivative was more rapid than that of the colored one. The presence of initial oligonucleotide derivative in the fullerene conjugate preparation was estimated roughly at about 10−20%. Digestion of agarose with β-agarase from Ozyme-New England Biolabs was performed according to the manufacturer's manual. Purified conjugates were precipitated with ethanol/sodium acetate solution, washed by ethanol, and redissolved in water.

Gel-Retardation Electrophoresis: In a typical experiment, 0.6 pmol of both duplex strands (5′-^{32}P-labeled 5′-CCACTTTTTAAAAGAAAAGGGGGGACTGG-3′ and nonradioactive 5′-CCAGTCCCCCCTTTTCTTTTAAAAAGTGG-3′) and oligonucleotide−fullerene conjugate (200 pmol) were mixed in 10 μL of 0.05 M MES buffer/0.05 M NaCl/0.005 M $MgCl_2$, pH = 6, heated to 90 °C for 3 min, cooled, and incubated for at least 5 h at 4 °C to form the triplex. Glycerol (2 μL) with bromophenol blue and xylene cyanol were then added, and the samples were applied to 12% nondenaturing polyacrylamide gel prepared on the same MES buffer (pH = 6.0). The electrophoresis was carried out at 5 W at 8−12 °C. Results were analyzed with a PhosphorImager (Molecular Dynamics), with the ImageQuant 5.0 program.

Melting Curves: Denaturation experiments were carried out with a Kontron Uvicon 923 System with a thermostatted cuvette. The solution (600 μL) contained 1.3 μM concentration of each of the three strands in 0.01 M sodium cacodylate, pH = 6.0, 0.1 M NaCl, 0.005 M $MgCl_2$. Two or three heating-cooling cycles were performed at 0.2 °C/min from 5 °C to 85 °C. Optical density at 260 nm

was measured every minute. The curves were processed with Microsoft Excel.

Computational Methodologies: All calculations were performed with a Silicon Graphics Octane R12000 workstation. The molecular structure of triplex DNA was built up by using JUMNA molecular modeling software,[31] which allows the construction in standard conformation of a DNA triple-helix as long as a strand of 16 base pairs. The structure of the 29 bp DNA triplex was obtained by joining 13 base pair fragments built by JUMNA. Two base pairs (CT at 5′-end and TC at 3′-end) were added to the ends of a studied 29 bp sequence with the aim of avoiding molecular modeling end effects. The atomic coordinates used to construct hybrid fullerene−trimethoxyindole conjugates were obtained by energy-minimization calculations performed for the initial structure with the MOE (2001.01) modeling package.[32] For energy calculations, the AMBER94 force field[33] was applied and the macromolecule environments (counterions and water molecules) were taken into account by introducing a distance-dependent dielectric constant. The minimization was carried out by a variant of the conjugate gradient method, until the *rms* value was <0.1 kcal/mol/Å. Bis(functionalized) fulleropyrrolidine structures **14** and **24** were built and fully optimized without geometry constraints by RHF/AM1 semiempirical calculation.[34] Atomic charges were calculated by fitting to electrostatic potential maps (CHELPG method).[35] The Gaussian98 software package was used for all quantum mechanical calculations.[36] Hybrid fullerene−trimethoxyindole−oligonucleotide conjugates **1** and **3** were assembled using MOE software. All conjugate structures were optimized with the MMFF94 force field[37−41] implemented by MOE until the *rms* value of the conjugate gradient was < 0.1 kcal/mol/Å. During the optimization process, all the Cartesian coordinates of triplex DNA were frozen, permitting the bis(functionalized) fulleropyrrolidine moiety to relax around the duplex DNA structure. Partial atomic charges for the bis(functionalized) fulleropyrrolidine were imported from the G98 output files. To analyze the conformational space of the bis(functionalized) fulleropyrrolidine moiety around the oligonucleotide structure, we performed an exhaustive conformational analysis with the Stochastic Conformational Search algorithm (SCS)[42] implemented by MOE. The SCS method generates conformations by randomly sampling local minima of the potential energy surface. This method generates new molecular conformations by randomly perturbing the position of each coordinate of each atom in the molecule by some small amount, typically less than 2 Å, followed by energy minimization. This minimization is intended to relieve bad nonbonded contacts. During the SCS procedure, all the coordinates of triplex DNA were frozen. After SCS, we collected 55 conformations sorted by their conformational energies. The most stable conformers of both **1** and **3** derivatives were optimized with the MMFF94 force field implemented by MOE until the *rms* value of the conjugate gradient was < 0.1 kcal/mol/Å.

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[1] S. R. Wilson, in *The Fullerene Handbook* (Eds.: K. M. Kadish, R. S. Ruoff), John Wiley & Sons, New York, **2000**, p. 437−465.
[2] T. Da Ros, M. Prato, *Chem. Commun.* **1999**, 663−669.
[3] A. W. Jensen, S. R. Wilson, D. I. Schuster, *Bioorg. Med. Chem.* **1996**, *4*, 767−779.
[4] C. S. Foote, *Top. Curr. Chem.* **1994**, *169*, 347−363.
[5] D. Guldi, M. Prato, *Acc. Chem. Res.* **2000**, *33*, 695−703.
[6] J. L. Anderson, Y.-Z. An, Y. Rubin, C. S. Foote, *J. Am. Chem. Soc.* **1994**, *116*, 9763−9764.
[7] R. Bernstein, F. Prat, C. S. Foote, *J. Am. Chem. Soc.* **1999**, *121*, 464−465.
[8] H. Tokuyama, S. Yamago, E. Nakamura, T. Shiraki, Y. Sugiura, *J. Am. Chem. Soc.* **1993**, *115*, 7918−7919.
[9] Y. N. Yamakoshi, T. Yagami, S. Sueyoshi, N. Miyata, *J. Org. Chem.* **1996**, *61*, 7236−7237.
[10] E. Nakamura, H. Tokuyama, S. Yamago, T. Shiraki, Y. Sugiura, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2143−2151.
[11] A. S. Boutorine, H. Tokuyama, M. Takasugi, H. Isobe, E. Nakamura, C. Hélène, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2462−2465.
[12] Y. Z. An, C. H. B. Chen, J. L. Anderson, D. S. Sigman, C. S. Foote, Y. Rubin, *Tetrahedron* **1996**, *52*, 5179−5189.
[13] A. S. Boutorine, O. Baland, H. Tokuyama, M. Takasugi, H. Isobe, E. Nakamura, C. Hélène, in *Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; (Eds.: K. M. Kadish, R. S. Ruoff), The Electrochemical Society, Inc., Pennington, NJ, **1997**, vol. 5, pp. 186−196.
[14] J. W. Schewczyk, E. E. Baird, P. B. Dervan, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1487−1489.
[15] M. Bergamin, T. Da Ros, G. Spalluto, A. Boutorine, M. Prato, *Chem. Commun.* **2001**, 17−18.
[16] D. L. Boger, D. S. Johnson, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1438−1474.
[17] M. Maggini, G. Scorrano, M. Prato, *J. Am. Chem. Soc.* **1993**, *115*, 9798−9799.
[18] M. Prato, M. Maggini, *Acc. Chem. Res.* **1998**, *31*, 519−526.
[19] D. Boger, T. Ishizaki, H. Zarrinmayeh, P. Kitos, O. Suntornwat, *J. Org. Chem.* **1990**, *55*, 4499−4502.
[20] L. Lebeau, P. Oudet, C. Mioskowski, *Helv. Chim. Acta* **1991**, *74*, 1697−1706.
[21] D. G. Knorre, P. V. Alekseyev, Y. V. Gerassimova, V. N. Silnikov, G. A. Maksakova, T. S. Godovikova, *Nucleosides Nucleotides* **1998**, *17*, 397−410.
[22] T. S. Godovikova, V. F. Zarytova, T. V. Maltseva, L. M. Khalimskaya, *Bioorg. Khim.* **1989**, *15*, 1246−1252.
[23] T. Mukaiyama, R. Matsueda, M. Suzuki, *Tetrahedron Lett.* **1970**, *22*, 1901−1904.
[24] G. N. Grimm, A. S. Boutorine, C. Hélène, *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 1943−1965.
[25] C. Giovannangeli, N. T. Thuong, C. Hélène, *Nucleic Acids Res.* **1992**, *20*, 4275−81.
[26] C. Giovannangeli, S. Diviacco, V. Labrousse, S. Gryaznov, P. Charneau, C. Hélène, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 79−84.
[27] Y. Karton, B. Bradbury, J. Baumgold, R. Paek, K. Jacobson, *J. Med. Chem.* **1991**, *34*, 2133−2145.
[28] D. Muller, I. Zeltser, G. Bitan, C. Gilon, *J. Org. Chem.* **1997**, *62*, 411−416.
[29] L.-C. Lo, C.-H. L. Lo, K. D. Janda, D. B. Kassel, F. M. Raushel, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2117−2120.
[30] J. C. Hummelen, B. Knight, J. Pavlovich, R. Gonzalez, F. Wudl, *Science* **1995**, *269*, 1554−1556.
[31] R. Lavery, *Junctions and bends in nucleic acids: a new theoretical modeling approach* (Eds.: W. K. Olson, R. H. Sarma, M. Sundaralingham), Adenine Press, New York, NY, **1988**, vol. 3, pp. 197−211.
[32] Molecular Operating Environment (MOE 2001.01), Chemical Computing Group, Inc, 1255 University St., Suite 1600, Montreal, Quebec, Canada, H3B 3X3, **2001**.
[33] S. J. Weiner, P. A. Kollman, D. T. Nguyen, D. A. Case, *J. Comput. Chem.* **1986**, *7*, 230−252.
[34] M. J. S. E. Dewar, G. Zoebisch, E. F. Healy, *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

[35] C. M. Breneman, K. B. Wiberg, *J. Comp. Chem.* **1990**, *11*, 361−365.
[36] M. Frisch, G. W. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, V. Zakrzewski, J. Montgomery, R. E. Stratmann, J. C. Burant, S. Dapprich, J. Millam, A. Daniels, K. Kudin, M. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle, J. A. Pople, *Gaussian 98*, revision A.6 ed.; Gaussian, Inc., Pittsburgh, PA, **1998**.
[37] T. Halgren, *J. Comput. Chem.* **1996**, *17*, 490−519.
[38] T. Halgren, *J. Comput. Chem.* **1996**, *17*, 520−552.
[39] T. Halgren, *J. Comput. Chem.* **1996**, *17*, 553−586.
[40] T. Halgren, *J. Comput. Chem.* **1996**, *17*, 587−615.
[41] T. Halgren, R. Nachbar, *J. Comput. Chem.* **1996**, *17*, 616−632.
[42] D. M. Ferguson, D. J. Raber, *J. Am. Chem. Soc.* **1989**, *111*, 4371−4378.

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