Synthesis and Self-Assembly of an Oligonucleotide-Modified Cyclobutadiene Complex

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Summary: An oligonucleotide-functionalized cyclobutadiene complex and its hybridization to a double-stranded organometallic DNA-object are reported here.

We describe, in this communication, a proof of concept for creating nanoscale architectures. We utilize oligonucleotides to self-assemble organic or organometallic modules into precise arrangements. Here, we lay the groundwork with the incorporation of a cyclobutadiene complex into the sugar−phosphate backbone of DNA and its self-assembly into a hybridized dimer. The ability to control the design of novel nanoscale architectures encompasses the ultimate goal of materials chemistry.[1] Assembly of molecular building blocks utilizing noncovalently programmed recognition sites offers a biological solution to a synthetic problem. The programmability of DNA, in particular, makes it an exceptionally useful tool in assembling organic and inorganic modules into predefined orientations with respect to each other.[2] The groups of Mirkin,[3] Schultz,[4] Tour,[5] Rubin,[6] Ihara,[7] and Bergstrom[8] have successfully employed the concept of utilizing DNA in this manner.

A commonly overlooked, and limiting, aspect of DNA nano-architectural design is the covalent attachment of DNA to an organic or inorganic module. Both Mirkin[3] and Schultz[4] use a thiol linker to attach oligonucleotides to gold nanoparticles—a highly effective postsynthetic method for gold. A more desirable method would be one in which the organic moiety of interest is directly fed into the oligonucleotide synthesizer, making it an integral part of the DNA strand *during its synthesis*. Bergstrom's work provides an example of a nonbiogenic organic molecule being connected to DNA by an oligonucleotide synthesizer;[8] however, that approach was limited in that the organic was attached to the CPG resin upon which the oligonucleotide was synthesized. While several groups have incorporated coordination compounds[9] and other synthetic moieties[10] into oligonucleotides, we desired a conjugate that was robust, whose moiety did not interfere with hybridization, and which did not require postsynthetic modification. Thus, we sought to exploit the well-established phosphoramidite chemistry.[11] Phosphoramidites are valuable in the construction of oligonucleotides by treating the organic or inorganic molecule as a modified nucleotide.[12] The groups of Kool,[13] Thuong,[14] and Letsinger[15] have incorporated molecules into the sugar phosphate backbone using this method for a variety of applications; however, it has yet to be linked to constructing oligonucleotide-directed architectures. We generalize this concept by proposing the use of *any diol*, providing a virtually limitless library of available organic moieties for addition onto any oligonucleotide. Thus, we can

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(1) See the July 1999 issue of *Chem. Rev.*

(2) For reviews see: (a) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1848. (b) Niemeyer, C. M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 585.

(3) (a) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607. (b) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L, Mirkin, C. A. *Science* **1997**, *277*, 1078. (c) Mucic, R. C.; Storhoff, J. J.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 12674. (d) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959.

(4) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. *Nature* **1996**, *382*, 609.

(5) Cassell, A. M.; Scrivens, W. A.; Tour, J. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 1528.

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

(7) (a) Ihara, T.; Nakayama, M.; Murata, M.; Nakano, K.; Maeda, M. *J. Chem. Soc., Chem. Commun.* **1997**, 1609. (b) Ihara, T.; Maruo, Y.; Takenaka, S.; Takagi, M. *Nucleic Acids Res.* **1996**, *24*, 4273.

(8) Shi, J.; Bergstrom, D. E. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 111.

(9) (a) Barton, J. K.; Kumar, C. V.; Turro, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 6391. (b) Hurley, D. J.; Tor, Y. *J. Am. Chem. Soc.* **1998**, *120*, 2194. (c) Telser, J. Cruickshank, K. A.; Schanze, K. S.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 7221. (d) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L.; Chan, C.-L. *J. Am. Chem. Soc.* **1989**, *111*, 7226. (e) Bannwarth, W.; Schmidt, D. *Tetrahedron Lett.* **1989**, *30*, 1513. (f) Manchanda, R.; Dunham, S. U.; Lippard, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 5144. (g) Chen, C.-H. B.; Sigman, D. S. *J. Am. Chem. Soc.* **1988**, *110*, 6570. (h) Modak, A. S.; Gard, J. K.; Merriman, M. C.; Winkeler, K. A.; Bashkin, J. K.; Stern, M. K. *J. Am. Chem. Soc.* **1991**, *113*, 283. (i) Magda, D.; Miller, R. A.; Sessler, J. L.; Iverson, B. L. *J. Am. Chem. Soc.* **1994**, *116*, 7439.

(10) (a) Khan, S. I.; Grinstaff, M. W. *J. Am. Chem. Soc.* **1999**, *121*, 4704. (b) Wiederholt, K.; McLaughlin, L. W. *Nucleic Acids Res.* **1999**, *27*, 2487.

(11) (a) Caruthers, M. H. *Acc. Chem. Res.* **1991**, *24*, 278. (b) Caruthers, M. H. *Science* **1985**, *230*, 281.

(12) (a) Coleman, R. S.; Dong, Y.; Arthur, J. C. *Tetrahedron Lett.* **1993**, *34*, 6867. (b) Coleman, R. S.; Kesicki, E. A. *J. Am. Chem. Soc.* **1994**, *116*, 11636. (c) Coleman, R. S.; Pires, R. M. *Nucleic Acids Res.* **1997**, *25*, 4771. (d) Coleman, R. S.; Arthur, J. C.; McCary, J. L. *Tetrahedron* **1997**, *53*, 11191.

(13) Kool, E. T.; Breslow, R. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1617.

(14) (a) Thuong, N. T.; Chassignol, M. *Tetrahedron Lett.* **1988**, *26*, 5905. (b) Durand, M.; Chevrie, K.; Chassignol, M.; Thuong, N. T.; Maurizot, J. C. *Nucleic Acids Res.* **1990**, *18*, 6353. (c) Giovannangeli, C.; Montenay-Garestier, T.; Rougee, M.; Chassignol, M.; Thuong, N. T.; Helene, C. *J. Am. Chem. Soc.* **1992**, *113*, 7775.

(15) (a) Manikrao, S.; Wu, T.; Letsinger, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8768. (b) Letsinger, R. L.; Wu, T. *J. Am. Chem. Soc.* **1994**, *116*, 811. (c) Letsinger, R. L.; Wu, T. *J. Am. Chem. Soc.* **1995**, *117*, 7323. (d) Lewis, F. D.; Helvoigt, S. A.; Letsinger, R. L. *J. Chem. Soc., Chem. Commun.* **1999**, 327.

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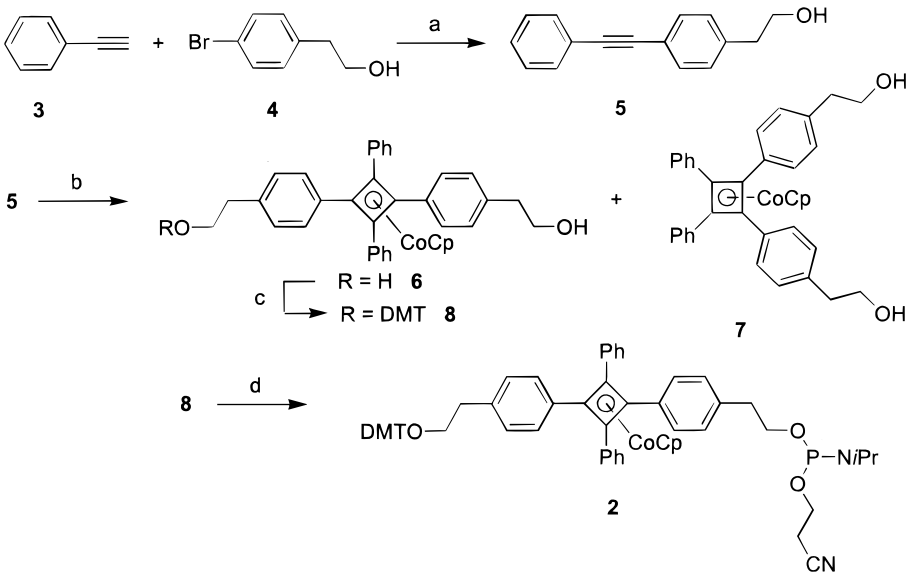
Scheme 1[a]



[a] Legend: (a) $PdCl_2(PPh_3)_2$/CuI/PPh_3/NEt_3; (b) $CpCo(CO)_2$/*p*-xylene; (c) DMTCl/pyr; (d) 2-cyanoethyl diisopropylchlorophosphoramidite/diisopropylethylamine/THF.

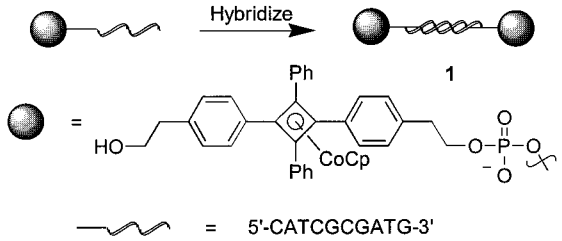


Figure 1. Schematic illustration of a duplex oligonucleotide-modified organic (OMO).

construct a wide variety of oligonucleotide-modified organic (OMO) molecules with tailored oligonucleotide sequences as well as tailored organic moieties for the construction of novel architectures applicable in materials chemistry.

As a first experiment, we desired to construct a dimer hybrid (**1**; Figure 1) of an OMO with a palindromic recognition sequence where the sphere can be any organic/organometallic molecule. In this specific case a (tetraphenylcyclobutadiene)cyclopentadienylcobalt complex was chosen. Thus, complex **2** was the target molecule. It contains a DMT protecting group and a phosphoramidite activating group, making it conducive for automated oligonucleotide synthesis. Preparation of the complex (Scheme 1) began with the Pd/Cu coupling[16] of phenylacetylene (**3**) with 4-bromophenethyl alcohol (**4**). Cyclization of the resulting substituted tolane (**5**) with cyclopentadienylcobalt dicarbonyl ($CpCo(CO)_2$) gave a mixture of isomers designated *ortho* and *para* with regard to substitution around the cyclobutadiene ring. These isomers were separable by chromatography.[17] The *para* isomer was subjected to DMT protection,[18] and the isolated monoprotected product (**8**) was activated with 2-cyanoethyl diisopropylchlorophosphoramidite.[11,18] The target molecule **2** was isolated and stored desiccated in the freezer.

The cyclobutadiene complex **2** was attached to the 5′-end of an oligonucleotide with a palindromic sequence of 5′ GTAGCGCTAC 3′. The sequence was synthesized on an automated oligonucleotide synthesizer and allowed to begin a cycle for the addition of another base. Following the activation step, a THF solution of the cyclobutadiene complex was syringed through the column containing the growing oligonucleotide. After a 2 min addition, the column was placed back online to complete the capping and oxidation steps before workup. Additionally, an oligonucleotide of the same sequence was synthesized without an organic for use as a control.

Purification of the cyclobutadiene OMO by HPLC allowed the isolation of two components. The UV−vis spectrum of the less retained component was characteristic of DNA with a broad band at 260 nm, while the UV−vis spectrum of the second component consisted of the broad 260 nm band with a shoulder at 310 nm, suggesting that this was the fraction containing the cyclobutadiene OMO. Figure 2 depicts the spectrum of the cyclobutadiene OMO compared with the spectra of the oligonucleotide control and the free cyclobutadiene complex (**6**).[19] Comparing the control spectrum with the cyclobutadiene spectrum, one can easily see that the additional shoulder at 310 nm must be due to the cyclobutadiene complex, as this feature is present in the spectrum of **6**. Electrospray MS of the cyclobutadiene OMO provided further evidence for covalent attachment. A single component of *m*/*z* 3657 was observed for the cyclobutadiene OMO, where as the control had a single component of *m*/*z* 3027—a difference of 640 Da, which can be accounted for by the cyclobutadiene

(16) Zhang, Y.; Schuster, G. B. *J. Org. Chem.* **1997**, *59*, 11855.

(17) Conclusive *ortho*/*para* isomer assignments await X-ray crystallography data. However, given that the *ortho* isomer should have a greater dipole moment than the *para* isomer, the faster running spot on TLC was assigned the *para* isomer. This was confirmed with ^{13}C NMR; the spectra of the DMT-protected/phosphoramidite-activated forms of each isomer were compared. There were four cyclobutadiene peaks distinguishable for the *ortho* isomer and only three for the *para* isomer (C_2 symmetry).

(18) *Oligonucleotides and Analogues: A Practical Approach*; Eckstein, F., Ed.; IRL Press: Oxford Press: New York, 1991.

(19) Note that the units of the *y* axis are not directly comparable, since the oligonucleotide spectra were acquired in aqueous solvent while the spectrum of **6** was taken in acetonitrile.

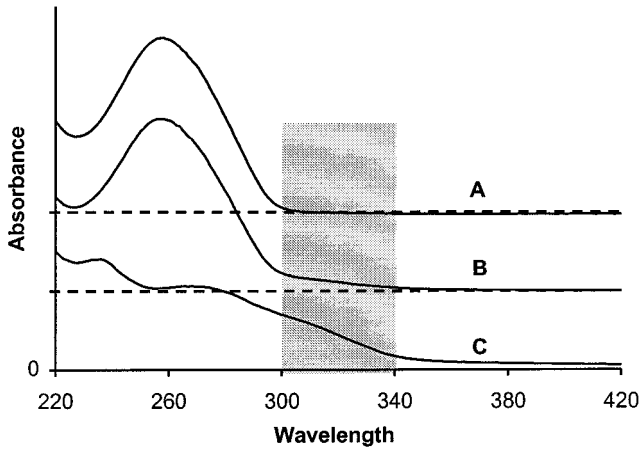


Figure 2. UV−vis spectra of DNA (**A**), cyclobutadiene OMO (**B**), and cyclobutadiene complex (**C**). The shaded area indicates the feature of the cyclobutadiene complex absent in the DNA but present in the cyclobutadiene OMO. The dotted baselines are added for clarity.

moiety. If the cyclobutadiene complex were noncovalently associated with the oligonucleotide, one would expect to see a molecular ion for just the oligonucleotide, which is not the case.

The cyclobutadiene OMO was annealed to form a double-stranded DNA hybrid, as shown in Figure 1. The double-stranded OMO (**1**) was characterized by melting temperature (T_m) and gel electrophoresis. The presence of the cyclobutadiene complex on the 5′-end of the DNA may influence duplex formation; thus, a melting-temperature study of the OMO and the DNA was performed from 20 to 80 °C. The melting temperatures as well as the shape of the curves were similar for each sample, demonstrating that the cyclobutadiene complex neither contributes to nor prevents the duplex formation of the double-stranded OMO.

A native polyacrylamide gel electrophoresis experiment was performed to compare the structure of the double-stranded OMO (**1**) with that of the duplex control (Figure 3). The nondenaturing gel demonstrated that the mobility of the OMO was less than that of the identically sized fragment without the complex attached. The mobility through the gel is a function of size, shape, and charge on the molecule. Thus, when the size of the OMO is compared with that of the unmodified DNA and the size markers, the OMO is slightly larger than the unmodified DNA. The covalently attached

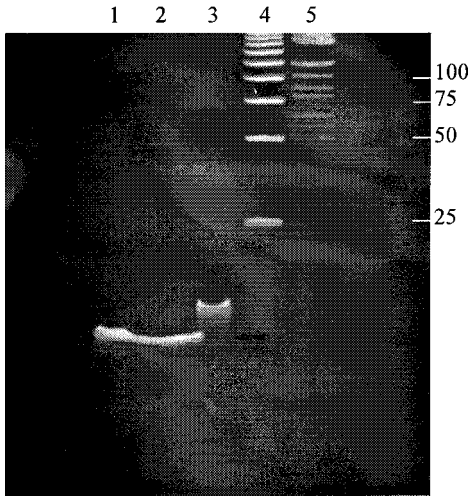


Figure 3. A 12% native polyacrylamide gel run at 600 V for 3 h in 1X TBE buffer stained with ethidium bromide (lanes 1 and 2) DNA control; (lane 3) cyclobutadiene OMO; (lane 4) standard 25 bp PCR ladder (sizes indicated); (lane 5) standard pBR322 HAE III digest.

cyclobutadiene complex influences thus the size and shape of the OMO.

This synthetic approach can be applied to *any* oligonucleotide sequence and *any* organic or organometallic module. The only prerequisite is the presence of the diol moiety and sufficient solubility of the DMT-protected/phosphoramidite-activated module in THF. The addition of the module to the oligonucleotide is not limited to the end of the sequence but, rather, can be incorporated site-specifically via the deprotection of the DMT group and continuation of sequence. Any available building block of interest for complex nanoscale architectures can be incorporated into DNA strands with minimum effort and high reliability. Soon we will report upon self-assembled nanostructures utilizing this concept.

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Supporting Information Available: Text giving experimental details and spectroscopic data for **2**−**8**, electrospray MS data, annealing details, melting curves, and PAGE details for dsOMO and dsDNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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