DNA Superstructures

Ru(II) Tris(bipyridyl) Complexes with Six Oligonucleotide Arms as Precursors for the Generation of Supramolecular Assemblies

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Nucleic acids can assemble into higher-order structures on the basis of complementary Watson–Crick base pairing. This self-assembly process has been exploited by a number of researchers[1–4] in the "bottom-up" approach to the generation of nonbiological structures of nanoscale dimensions.[5] In some studies, these assemblies have been based solely upon complementary DNA hybridization in which branch points are created by double-crossover structures[6,7] (generated as immobile junctions[8–10]) that are similar to those of DNA recombination intermediates; higher-order DNA networks can be made by this approach.[11,12] In other cases, DNA sequences have been tethered to multifunctional organic cores[13,14] or inorganic complexes[15–19] that are capable of self-assembling into more-complex networks such as dendrimers.[20,21] Oligonucleotides have been tethered to gold nanoparticles,[2,22,23] and complementary hydrogen bonding resulted in the formation of large assemblies that were used for diagnostics.[24,25] Nucleic acids can also be functionalized by conjugation, and this approach has led to the development of nanostructures and devices,[26,27] DNA–protein conjugates,[24] and the assembly of two- and three-dimensional networks. DNA-based nanoscale assemblies have been used for the construction of nanowires.[28,29] Herein we describe the preparation of six-oligonucleotide-armed ruthenium(II) tris(bipyridyl)-centered complexes as precursors for the generation of supramolecular nanoscale assemblies.

In the present monomer design, we chose $[Ru(bpy)_3]^{2+}$ with a Ru^{II} ion bound to three bipyridine (bpy) ligands as a core, and the DNA arms were tethered at the 4- and 4′-positions of each bipyridine ligand (Figure 1). Although $[Ru(bpy)_3]^{2+}$ exists in two enantiomeric forms, substitution at all three 4 and 4′ sites results in an octahedral arrangement of the substituents in both enantiomers. First, enantiomerically pure DNA–$[Ru(bpy)_3]^{2+}$ conjugates should be attainable by appropriate chromatographic resolution of the Ru complexes as has been described for related ruthenium complexes,[30–32] followed by their incorporation into DNA conjugates. However, such chromatographic separations are not yet routine, and successful isomer resolution still depends in part upon the nature of the complex. The choice of sequences

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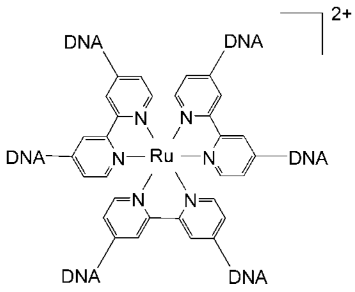


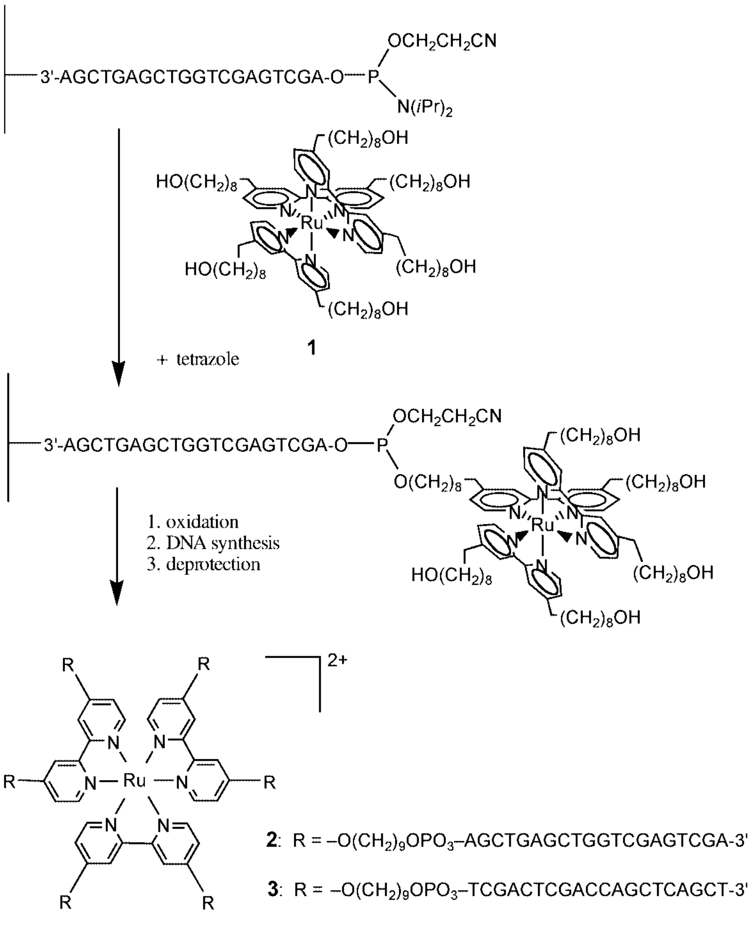
Figure 1. A $[Ru(bpy)_3]^{2+}$ center with six DNA sequences tethered at the 4 and 4′ positions.

for the present building blocks is quite general, but self-assembly of monomers into higher-order structures requires either monomers that tether self-complementary sequences, or two or more monomers that tether complementary sequences.

Modeling studies suggested that a linker was required between the octahedral core and the DNA duplex to reduce steric crowding between the six termini of the tethered duplexes. A Ru^{II} complex **1** with six nine-carbon-atom linkers was prepared and then incorporated into a single, support-bound DNA sequence (Scheme 1). Subsequent extension of the remaining five linkers by solid-phase DNA synthesis completed the assembly of the monomers (Scheme 1). To generate a complex with six arms of the same sequence and uniform polarity, we synthesized the initial sequence on a solid support in the conventional 3′ to 5′-direction (Scheme 1). The Ru^{II} complex was then incorporated into the support-bound 20-mer by a reverse-coupling protocol.[33,34] Synthesis of DNA in the unconventional 5′ to 3′-direction by using the reverse nucleoside monomers[35] (3′-dimethoxytrityl, 5′-phosphoramidites) allowed the functionalization of the remaining five linkers at the metal complex. During this latter process, the reaction times for the coupling reactions were extended to 60 min. With six hydroxy-terminated linkers present in **1** (see Scheme 1), it is conceivable that cross-linking of the synthesis support might occur; that is, one Ru^{II} complex could react with two (or more) support-bound DNA sequences. We believe this to be, at most, a minor reaction pathway, but for the described complexes it is also relatively unimportant with respect to the nature of the final product. Because all six sequences attached to the Ru tris(bipyridyl) center are identical both in sequence and polarity, complexes formed by either a single coupling or a cross-linking event to the support-bound sequence are all identical after deprotection. Control reactions with the Ru tris(bipyridyl) complex confirmed that $[Ru(bpy)_3]^{2+}$ was stable to the basic conditions encountered during the deprotection of the DNA sequences.

The desired six-arm complexes **2** and **3** were purified by a combination of HPLC and PAGE (polyacrylamide gel electrophoresis) techniques. The reversed-phase HPLC traces typically resulted in three peaks (see Supporting Information). The third peak (37.9 min) corresponded to an orange-colored species and contained the product, namely **2** or **3** (see Scheme 1). The first peak (22.7 min) contained DNA without any ruthenium which resulted from the growth of DNA strands that had not coupled with the Ru complex **1**, whereas the second peak (33.1 min) remains unidentified. The desired complexes were isolated by HPLC with a C_{18} fast-flow Poros column, which gave essentially the same peak pattern as the analytical HPLC traces but broader peaks.

Isolation by PAGE was necessary to resolve the 5-arm and 6-arm DNA–$Ru(bpy)_3$ monomers because the eluted fraction corresponding to the third peak during HPLC isolation comprised both species. The identity of the two conjugates was tentatively assigned from their relative migration in the gel, and this assignment was confirmed by using a hybridization assay (see below). The ratio of the 6-arm to 5-arm complex varied for each synthesis, but it was always at least 2:1. Yields of the 6-arm complex varied typically from 5–10 A_{260} units (absorbance units measured at 260 nm). This relatively low yield is at present largely a result of the need to use wide-pore (2000 Å) supports in the synthesis of the DNA; the initial DNA loading on these supports was generally no more than 8 $\mu mol\,g^{-1}$. Furthermore, the reverse coupling step used to incorporate the Ru tris(bipyridyl) complex **1** into the DNA strand is not the high yielding reaction typical of phosphoramidite couplings. The six-arm DNA–Ru^{II} complex was characterized by MALDI-



Scheme 1.

TOF mass spectrometry and UV/Vis and fluorescence spectroscopies.

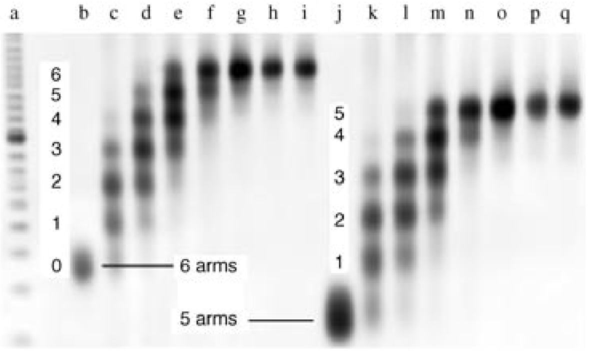


Figure 2. Nondenaturing PAGE analysis of DNA–$[Ru(bpy)_3]^{2+}$ conjugates (≈25 nM) that result from the synthesis of **3**. Left panel, hybridization results for the 6-arm conjugate; right panel, hybridization results for the 5-arm conjugate. Lane a: 20 bp ladder; lane b: isolated 6-arm conjugate; lanes c–i: 6-arm conjugate with increasing amounts of the complementary 20-mer; lane j: isolated 5-arm conjugate; lanes k–q: 5-arm conjugate of lane 10 with increasing amounts of the complementary 20-mer. bp = base-pairs.

The number of DNA arms present in the isolated Ru-centered monomers was confirmed by stepwise hybridization with the complementary 20-mer (Figure 2). The desired six-arm complex (Figure 2, left panel) exhibited six hybridization products, each leading to a stepwise reduction in the mobility. The hybridization bands on the gel are thought to correspond to six-arm-monomer complexes that have from one to six arms hybridized to the complementary 20-mer. Besides the identified six-arm complex, a second complex with slightly greater electrophoretic mobility was also isolated. The hybridization assay of this complex with the complementary 20-mer indicated that this material contained only five arms (Figure 2, right panel). These hybridization assays not only confirm the number of arms present, but they also confirm the ability of the arms to participate in hybridization reactions and exist as duplexes. Thermal melting studies were less helpful in the characterization of the hybridization products as a single transition resulted for all six duplexes.

Table 1: Migration Anomalies for $[Ru(bpy)_3]^{2+}$ Complexes that Tether up to Six DNA Duplexes.[a]

DNA Duplex Arms	0	1	2	3	4	5	6
Sequence Size	120	140	160	180	200	220	240
Apparent Size[b]	135	190	270	355	453	511	638
Migration Anomaly[c]	1.13	1.36	1.69	1.97	2.27	2.32	2.66

[a] Ratio of acrylamide to bisacrylamide 75:1. [b] Apparent size relative to a 20 bp standard ladder. [c] Migration anomaly = apparent size/sequence size.

The PAGE analyses of the DNA hybridization products in Figure 2 were complicated by migration anomalies. Migration of the hybridized complexes was only effective upon reduction of the cross-linking in the gels by using a 75:1 acrylamide:bisacrylamide mixture in place of the more conventional 19:1 mixture. However, even with reduced cross-linking, and presumably the presence of larger gel pores, the complexes appeared to migrate anomalously. The complex with six single-stranded arms exhibited a minimal anomaly of 1.13 (Table 1, 0 duplex arms): a moderate effect which suggests that the complex is still relatively flexible and that the geometry of placing six DNA arms about a central core does not significantly alter the nature of the gel migration. That is, the complex appears to be flexible enough to pass through the pores in a similar fashion to single- or double-stranded (ss or ds) DNA even though the geometry of the DNA complex is more starlike rather than linear. The observed small anomaly might be explained by the presence of the ruthenium metal ion and the additional mass of the bipyridine ligands and the six nine-carbon-atom linkers. However, the migration anomaly becomes greater with increasing numbers of hybridized arms; that is, as the single-stranded sequences are converted into duplexes, the complexes migrate more slowly than expected for the lengths of the sequences. One 20-mer hybridization event increases the size of the initial 120-residue complex to 140 residues, but the 140-mer complex migrates as a 190-mer (a migration anomaly of 1.36). With each subsequent hybridization event the sequence size increases by 20 residues, but the apparent size increases by 110, 175, 253, 291, and 398 residues relative to its actual size. The fully hybridized complex is a 240-mer but it migrates as a 638-mer—a migration anomaly of 2.66 (Table 1). This phenomenon may reflect a greater extent of entanglement in the gel fibers by the "starshaped" Ru^{II}–bpy–DNA-duplex complexes relative to the linear shapes of ss and dsDNA or even relative to the more flexible nature of the single-stranded DNA–Ru conjugates. The dramatic migration anomalies observed with an increase in the number of duplexes tethered to the Ru tris(bipyridyl) center suggest that the hybridization events generate a complex that displays a greater rigidity than the unhybridized six-stranded complex; the more rigid the multi-arm complex, then the more entanglement there will be between the complex and the gel, and thus the more difficulty the complex will have in migrating through the matrix. The rigidity of the monomer has been suggested[4] as an important parameter for the formation of higher-order DNA assemblies.

In this first study of a Ru center that tethers six DNA strands, we employed a nine-carbon-atom linker between the DNA duplexes and the $[Ru(bpy)_3]^{2+}$ center. The linker and the $[Ru(bpy)_3]^{2+}$ moiety taken together have a maximum extension of ≈15 Å from the center of the complex, whereas the diameter of the duplex DNA is ≈20 Å. The length of the linker may introduce some floppiness to the system, but even at this length, rotation of the DNA duplexes about the linker relative to one another can still result in significant restrictive steric or electronic effects with neighboring helices. To examine a more restrictive system, we prepared the $[Ru(bpy)_3]^{2+}$ center with six hydroxyethyl (two-carbon-atom) linkers and attempted to prepare the corresponding complex that tethers six DNA sequences. However, we were unable to obtain any material that contained all six DNA sequences tethered to the Ru–bipyridyl center. The optimal length of the linker for complexes of this type therefore lies between the 9-carbon-atom linker with which the target complex was obtained and the 2-carbon-atom linker that was unsuccessful.

A second hybridization assay (in an agarose gel) was performed with the monomers **2** and **3** (see Scheme 1), each of which contained six identical DNA arms that were complementary to those of the other monomer. As size markers, the respective hybridization products of **2** and **3** with six equivalents of the corresponding complementary 20-mer were used. These samples (Figure 3, lanes a and e, respectively) each provided a species with 240 residues (6 × 20 bp) that binds ethidium bromide to yield intensely fluorescent bands in the gel. The hybridization assay was performed with varying ratios of **2**:**3**. When both **2** and **3** were present, much of the resulting DNA was present as high-molecular-weight assemblies that did not migrate from the gel wells (Figure 3). In the presence of ethidium bromide, the DNA in the gel wells was intensely fluorescent which suggests the presence of conventional duplexes that are capable of intercalative binding. When either of the monomeric complexes was present in excess (Figure 3, lanes b and d), the excess unhybridized monomer was observed at the bottom of the gel with a greater mobility than those of the hybridized standards present in lanes a and e. However, the fluorescence intensity of the starting materials was significantly reduced relative to the standards in lanes a and e because they contain single-stranded arms that do not bind ethidium bromide as effectively. These observations suggest that the high-molecular-weight assemblies in lanes b–d were not simply nonspecific aggregates. The absence of any low-molecular-weight species (dimers, trimers, etc.) in lanes b–d (Figure 3) may indicate that the assembly process, which involves multiple hybridization events for each monomer, may mimic crystallization processes in which the monomers readily assemble into a macroscopic system after initiation by a seed structure. We cannot determine at this time whether the assemblies formed by hybridization have an ordered (i.e., cubic) geometry or whether they contain significant amounts of linear hybridization products or other types of nonspecific assemblies such as those resulting from interpenetration.

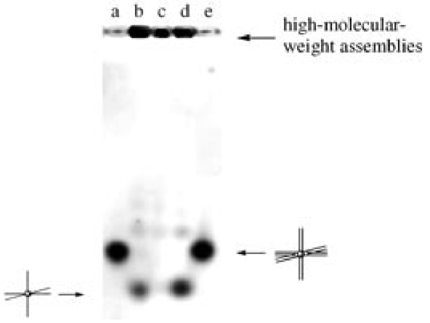


Figure 3. Hybridization of **2** and **3** as visualized by ethidium bromide after agarose gel electrophoresis. Lane a: **2** + excess 20-mer; lane b: **2** + **3** (2:1); lane c, **2** + **3** (1:1); lane d: **2** + **3** (1:2); lane e, **3** + excess 20-mer.

In conclusion, this study suggests that multi-arm DNA complexes might be useful for the assembly of regular DNA lattices of nanoscale dimensions.

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