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Six-Arm Oligonucleotide Ru(II)-Tris(bipyridine)-Centered Complexes as Precursors for the Generation of Supramolecular Periodic Assemblies

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Materials and Methods

Materials

Reagents and solvents were from Aldrich (St. Louis, MO), Strem Chemicals (Newburyport, MA), and Lancaster (Windham, NH). Thin-layer chromatography was performed using aluminum-backed precoated aluminum oxide 60 F_{254}, neutral plates from EM Science (Gibbstown, NJ). Preparative chromatography was performed using aluminum oxide, activated, neutral, Brockmann I standard grade from Aldrich and Alumina G-F preparative plates from Analtech (Newark, DE). ^{1}H NMR spectra were obtained on 300 MHz and 400 MHz Varian multiprobe spectrometers. High resolution electrospray mass spectroscopy was performed on a Micromass LCT system.

Oligonucleotides were synthesized on an Applied Biosystems 394 DNA/RNA synthesizer (Foster City, CA). DNA synthesis grade acetonitrile was purchased from Burdick and Johnson (Muskegon, MI). DNA synthesis reagents, 2000 Å 3'-terminal nucleotide CPG support, chemical phosphorylation reagent, tri(ethylene glycol) phosphoramidite, 3'-cyanoethyl (CE) phosphoramidites and 5'-CE phosphoramidites were obtained from Glen Research (Sterling, VA). 5' 1,000 Å CPG supports came from Biosearch Technologies (Foster City, CA).

High performance liquid chromatography was performed on a Waters 600E Multisolvent Delivery System equipped with a Waters 2487 dual wavelength absorbance detector (Milford, MA). Purification was performed on a 4.6mmD/100mmL PEEK column containing POROS Oligo R3 reverse phase support purchased from Applied Biosystems (Framingham, MA). HPLC grade acetonitrile and methanol was obtained from Fisher Scientific (Fair Lawn, NJ).

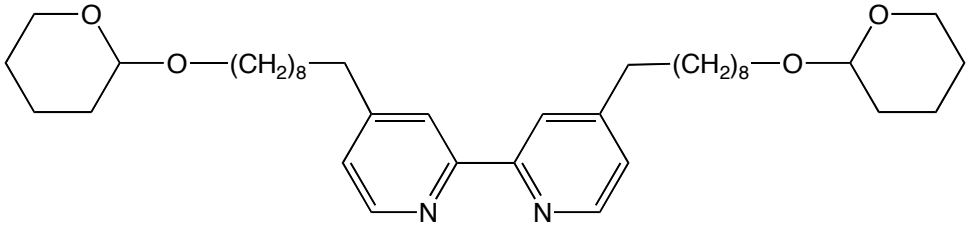
UV-Vis measurements were obtained on a Beckman DU640B spectrophotometer (Fullerton, CA). Polyacrylamide gel electrophoresis (PAGE) apparatus was from Hoefer

Scientific. Agarose gel electrophoresis was performed on a Sub-cell GT system purchased from Bio-Rad (Hercules, CA). Ethidium bromide and the 20 base pair standard were obtained from Sigma (St. Louis, MO). Imaging of PAGE and agarose gels was conducted on a BioRad Molecular Imager FX system equipped with Quantity One software.

MALDI-TOF mass spectrometry data for metal complex-DNA conjugates were obtained from the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, IL.

Methods

Synthesis of the metal complex **(1)**



4

4,4'-Bis-[9-(tetrahydro-pyran-2-yloxy)-nonyl]-[2,2']bipyridinyl (4). 8-Bromo-1-octanol was either purchased from Aldrich or synthesized by literature methods.[1] 8-bromo-1-[(tetrahydropyran-2'-yl)oxy]octane was purchased from Lancaster or synthesized based on a method described by Jayasuriya et al.[2] The synthesis of **4** was adapted from a synthesis described by Modak et al. for the synthesis of a 4'-methyl-4-carbon linker substituted bipyridine.[3] 1.71 g (9.28 mmol) of 4,4'-dimethyl-2,2'-bipyrdine was taken up in 75 mL anhydrous THF and cooled on an ice bath to 0 ºC. 2.0 M lithium diisopropyldiamide (LDA) (10.2 mL, 20.4 mmol) was added dropwise with stirring. The reaction was stirred at 0 ºC under inert conditions for 4 h. 6.0

g (20.4 mmol) of 8-bromo-1-[(tetrahydropyran-2'-yl)oxy]octane in 25 mL anhydrous THF was then added dropwise with stirring and the reaction was allowed to warm to ambient temperature. After stirring overnight, TLC on alumina eluted in 2.5% MeOH in CH_2Cl_2 appeared to show that the reaction had proceeded, although it was difficult to say definitively since the starting material and product have very close R_f values. The reaction was quenched by the addition of H_2O. CH_3Cl was added and the organic mixture was washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and evaporated to yield a yellow-brown oil. Purification on alumina eluted in 10% EtOAc in hexane yielded 4.59 g (7.54 mmol, 81.3%) of a pale yellow oil. R_f (alumina, 2.5% MeOH/CH_2Cl_2): 0.80. ^{1}H NMR (400 MHz, $CDCl_3$): 8.59 (d, 2H, Ar H6, H6'), 8.26 (s, 2H, Ar H3, H3'), 7.16 (d, 2H, Ar H5, H5'), 4.61 (t, 2H, O-CH-O), 3.96-3.38 (m, 8H, THP-O-CH_2-, CH_2), 2.72 (t, 4H, CH_2-Ar), 1.90-1.25 (m, 40 H, $(CH_2)_7$, $(CH_2)_3$) ppm.

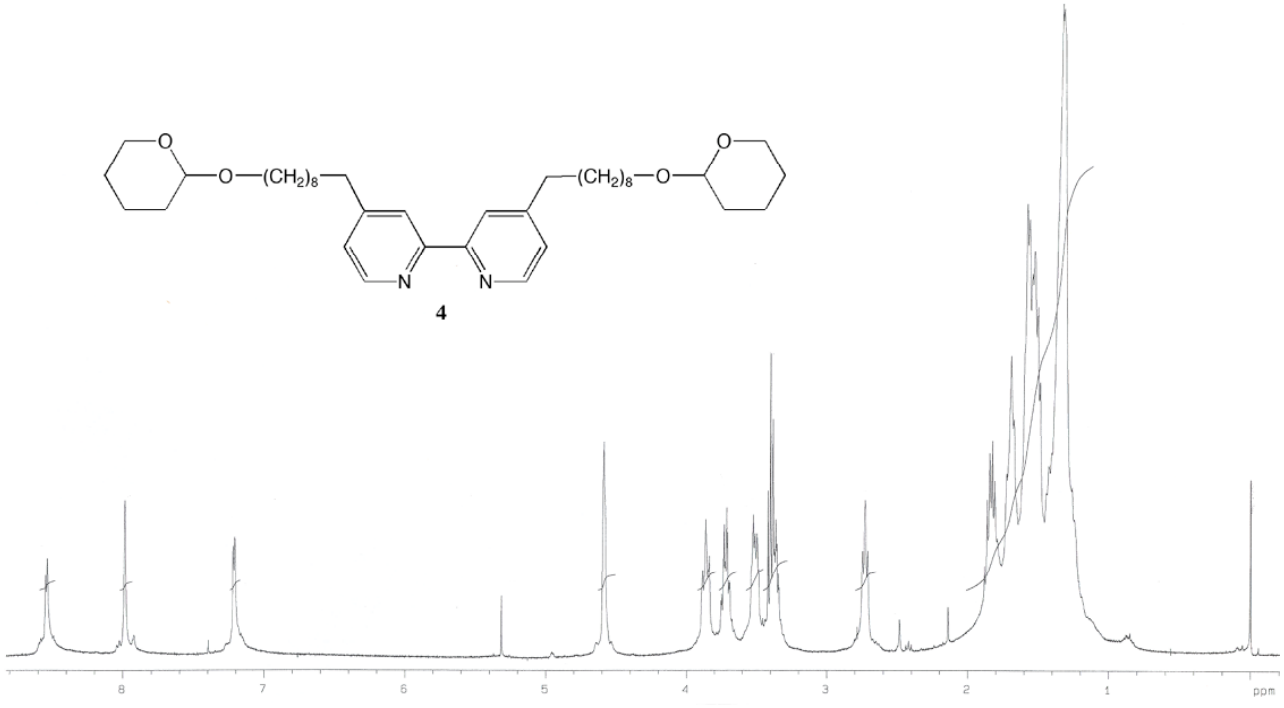
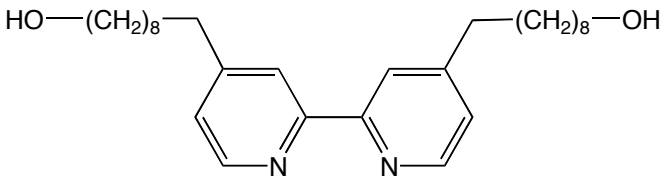


Figure S1. 400 Mhz 1H-NMR of compound **4** in $CDCl_3$.



5

9-[4'-(9-Hydroxy-nonyl)-[2,2']bipyridinyl-4-yl (5)

To 4.59 g of **4** was added 50 mL THF and 50 mL 25% HCl. The reaction was allowed to proceed overnight and went from clear yellow to cloudy. The reaction mixture was then poured into a flask, diluted with CH_3Cl, and quenched by the addition of solid Na_2CO_3. Once the reaction was quenched completely, the mixture was washed thoroughly with water, dried over Na_2SO_4, filtered and evaporated to yield yellow oil. Treatment of the yellow oil with a small amount of CH_2Cl_2 and hexane gave 1.75 g of a white solid (3.98 mmol, 52.8%). m.p.: 115-118 ^oC. R_f (alumina, 5% MeOH/CH_2Cl_2): 0.40. ^1H NMR (400 MHz, $CDCl_3$): □ 8.55 (d, H6, H6', 2H), 8.23 (s, H3, H3', 2H), 7.13 (d, H5, H5', 2H), 3.62 (t, CH_2-OH, 4H), 2.69 (t, -CH_2-Ar, 4H), 1.67-1.30 (m, CH_2, 28H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): □ 156.065, 153.349, 148.950, 124.207, 121.787, 62.979, 35.877, 32.972, 30.711, 29.711, 29.710, 29.657, 29.573, 26.077 ppm. HR-MS (ES+): expected (M +1Na^+): 463.3300, observed: 463.3307.

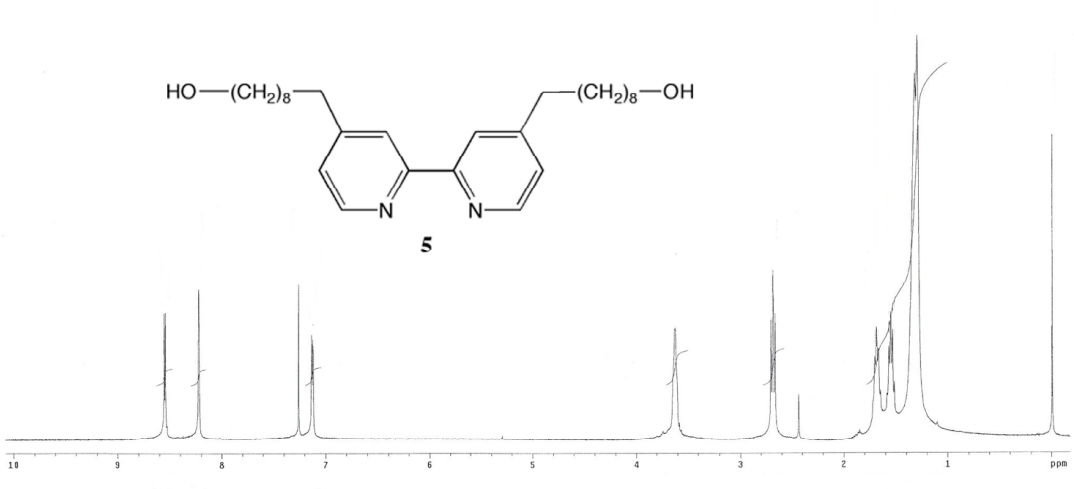
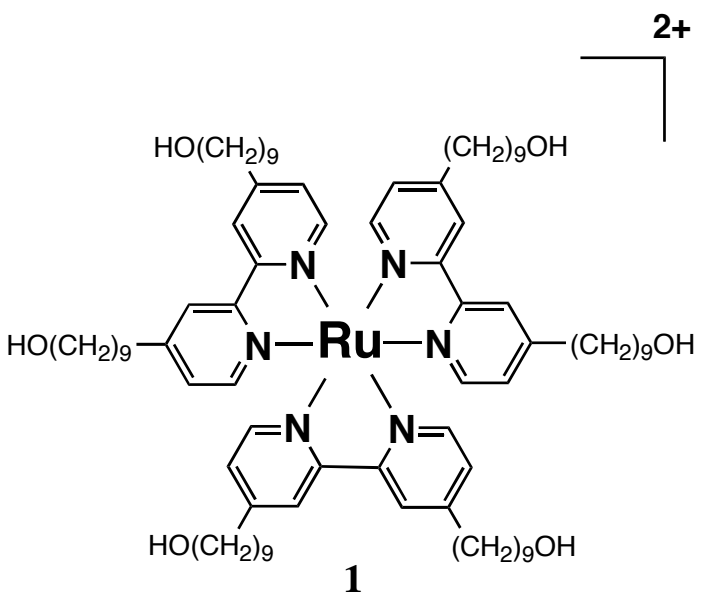


Figure S2. 400 MHz 1H-NMR of compound 5 in $CDCl_3$.



1

Ruthenium (II) tris(9-[4'-(9-hydroxy-nonyl)-[2,2']bipyridinyl-4-yl]-nonan-1-ol) hexaflurophosphate (1)

$Ru(DMSO)_4Cl_2$ was synthesized as previously described.[4] 0.250g (0.567 mmol) of bipyridine **5** and 0.0915g (0.189 mmol) of $Ru(DMSO)_4Cl_2$ was taken up in 25mL EtOH and heated to reflux. After 3 days, the reaction mixture was cooled and concentrated to approximately 3 mL. 0.037g NH_4PF_6 (0.227 mmol) was added and the solution was stirred for 0.5h. The remaining EtOH was evaporated and the product was purified on aluminum column eluted with 10% MeOH in CH_2Cl_2. The bright red-orange fractions were combined and evaporated to yield 0.267 g (0.156 mmol, 82.5%) of a deep red oil that formed a foam when placed under high vacuum. This material, isolated as a mixture of Ru diastereomers, migrated as a single spot by tlc ($CHCl_3/CH_3OH$ 9/1) HR-MS (ES+): expected (m/2+): 711.4633, observed: 711.4641. λ_{max}= 252 nm, 286 nm, 459 nm. ε_{260} = 19594 $cm^{-1}mM^{-1}$; ε_{460} = 15.893 $cm^{-1}mM^{-1}$.

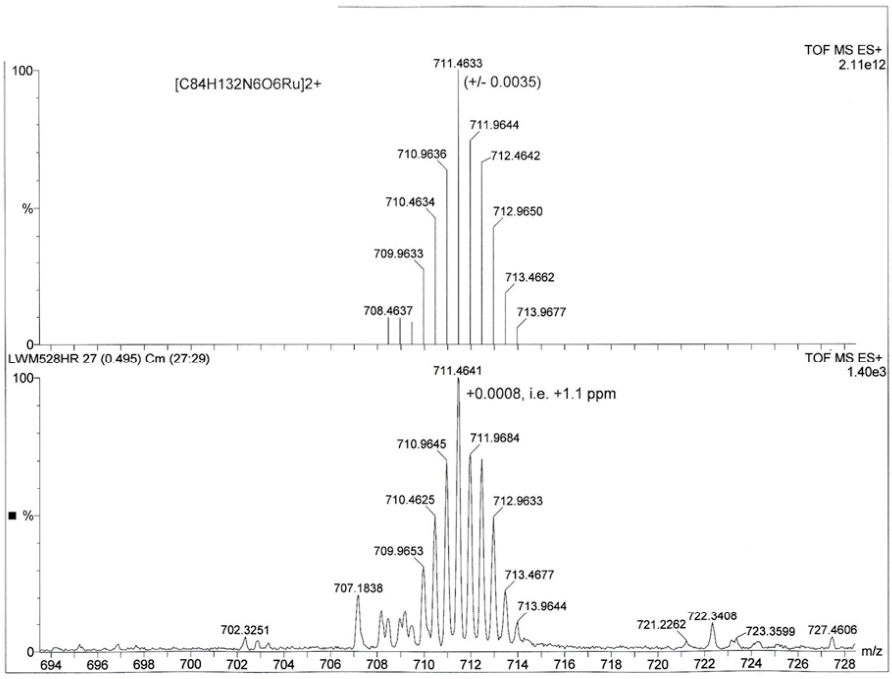
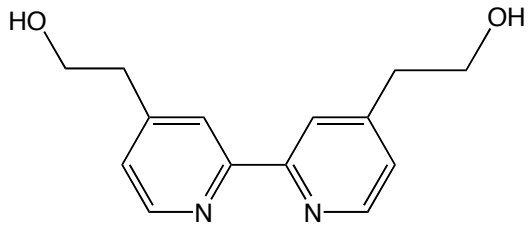


Figure S3. Theoretical high resolution TOF MS ES+ (above), experimental spectrum (below) of Ru-complex **1**.



6

4,4'-bis(2-hydroxyethyl)-2,2'-bipyridine (6). The procedure for synthesizing **4.1** was derived from a combination of procedures as reported by Kaes et al. and Ghosh and Spiro.[9,10] 4,4'-dimethyl-2,2'-bipyridine (1.00 g, 5.43 mmol) was taken up in 25 mL anhydrous

tetrahydrofuran (THF) and cooled to 0 ^{o}C. 10.86 mL of 2 M lithiumdiisopropylamide (LDA) in THF/heptane/benzene was diluted to 25 mL with anhydrous THF and added dropwise with stirring to the bipyridine solution. The reaction was gradually warmed to ambient temperature and stirred for 4 hours under inert conditions. After 4 hours, 2.61 g of paraformaldehyde was added. The reaction mixture turned from a deep brown to a pale orange color. The reaction was monitored by TLC (9:1 dichloromethane: methanol). After stirring overnight, the reaction was quenched with ice water and extracted with diethyl ether. A viscous, orange-brown oil was obtained upon concentration *in vacuo*. Purification was performed by column chromatography on alumina. Unreacted starting material and partially reacted product was eluted in 98:2 dichloromethane:methanol. The desired product **6** was eluted in 95:5 dichloromethane:methanol. Fractions containing the product were concentrated in vacuo to yield a yellow oil. After further purification on preparative alumina TLC plates (95:5 dichloromethane:methanol), a white solid was obtained (24.4%, 0.32 g). m.p.: 123-125 ^{O}C. R_f: 0.57 (alumina, 95:5 CH_2Cl_2:CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$): □ 8.42 (2H, d, H6,6'), 8.15 (2H, s, H3,3'), 7.13 (2H, d, H5,5'), 3.96 (4H, t, CH_2), 2.92 (4H, t, CH_2), 2.4 (2H, broad, OH) ppm; ^{13}C (400 MHz, CDCl3): d = 156.7, 150.373, 149.9, 125.7, 122.9, 63.6, 39.9 ppm; mp = 119-121 ºC; HR-MS (ES+): expected (M + H^+): 245.1290, observed: 245.1281.

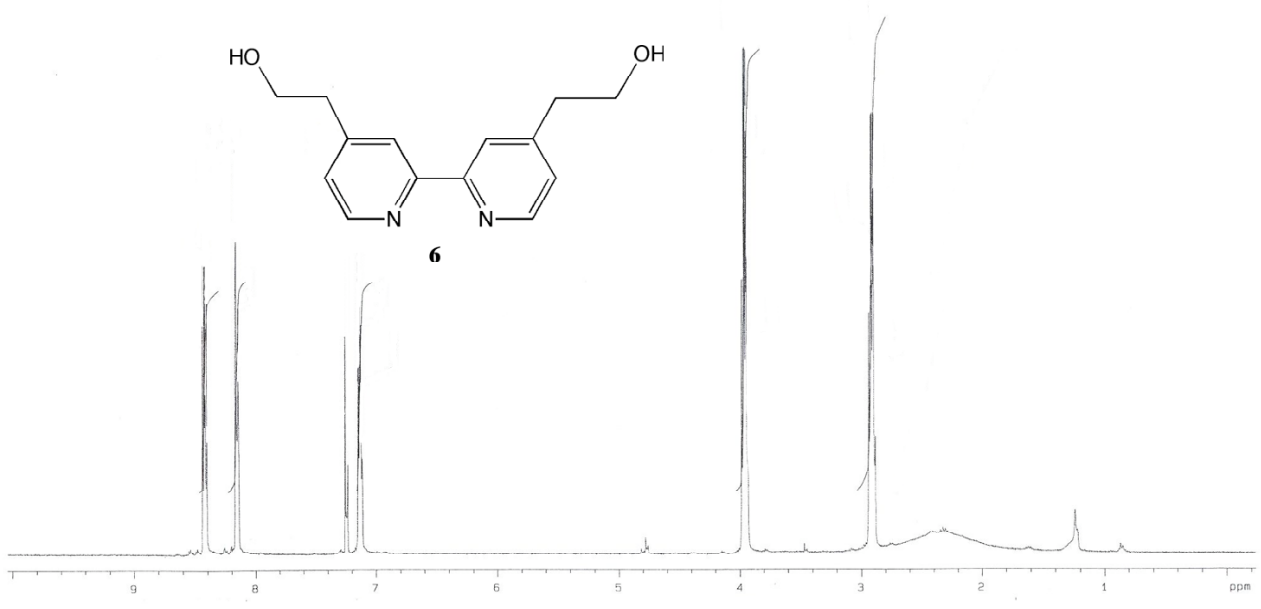
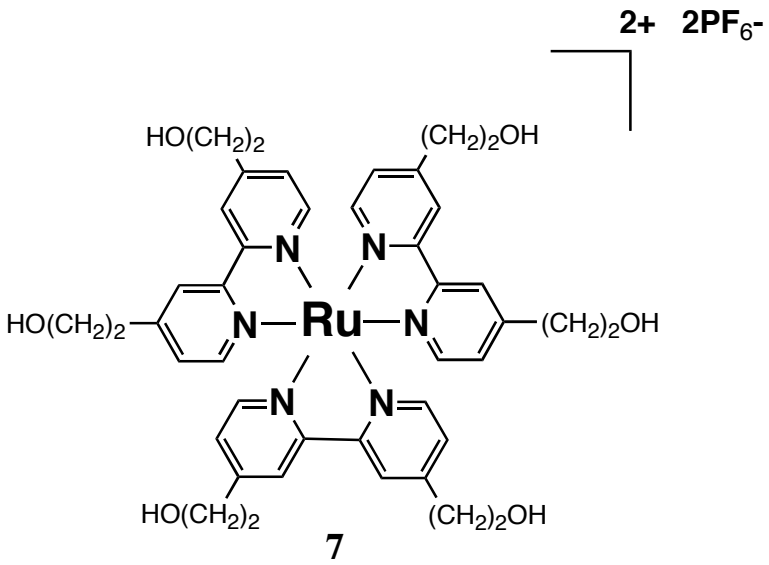


Figure S4. 400 MHz 1H-NMR of compound **6** in $CDCl_3$.



7

Ruthenium(II)tris(4,4'-bis(2-hydroxyethyl)-2,2'-bipyridine) hexafluorophosphate (7). $Ru(DMSO)_4Cl_2$ was synthesized according to literature procedures.[4] 4,4'-[bis(2-hydroxyethyl)-2,2'-bipyridine (**6**) (0.171g, 0.699 mmol) was dissolved in 10 mL of ethanol. To this solution was added $Ru(DMSO)_4Cl_2$ (0.103 g, 0.212 mmol). The mixture was heated to

reflux under nitrogen. After 1 hour, the solution turned from yellow to red-brown. After 7 days, the solution was cooled to ambient temperature and evaporated to dryness. The orange-red residue was taken up in water and filtered through cotton. Addition of excess NH_4PF_6 resulted in the formation of an orange-red oily precipitate. The water was decanted and the solid dried under high vacuum. The orange-red precipitate was treated with diethyl ether and filtered. The product was dried in a vacuum oven at 50 ºC (0.150 g, 62.8%). This material migrates as a single spot by tlc ($CHCl_2/CH_3OH$ 95/5). UV/Vis (ethanol): λ_{max} = 285 nm, 461 nm; Fluorescence: λ_{ex} = 280 nm, λ_{em} = 594 nm. HR-MS (ES+): expected (m/2+): 417.1340, observed: 417.1330.

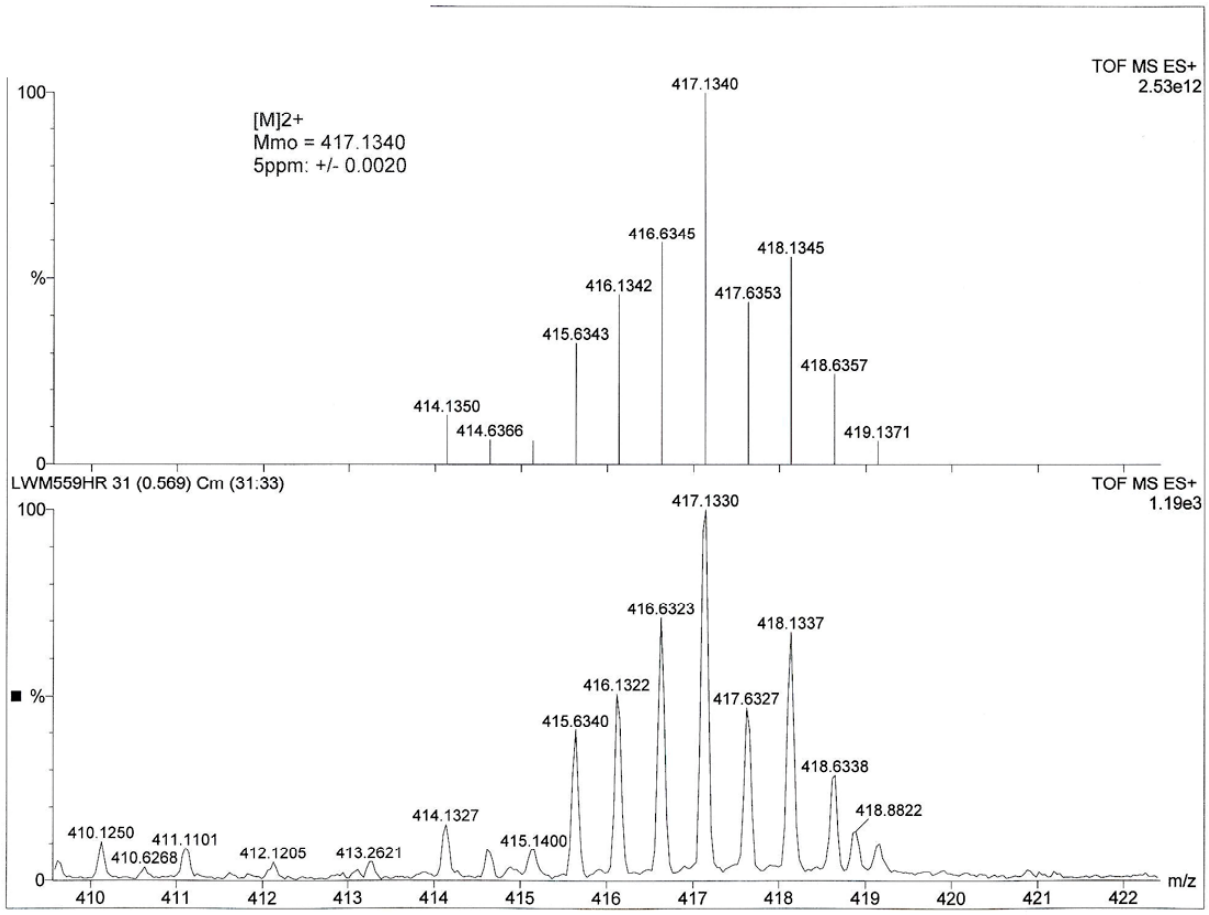


Figure S4. Theoretical high resolution TOF MS ES+ (above), experimental spectrum (below) for Ru complex **7.**

DNA synthesis

The initial DNA sequence was synthesized on a 1 μM scale on 2000 Å CPG but otherwise in the conventional manner.[5]

Introduction of the Ru complex and strand extension

The ruthenium complex was then introduced by using a reverse coupling technique.[6,7] 200 μL 2-(cyanoethyl)-tetraisopropylphosphodiamidite in 200 μL was drawn into a 1mL plastic syringe with a Luer fitting. 200 μL tetrazole activator solution was drawn into a second syringe. The syringes were attached to the ends of the DNA synthesis columns and the solutions mixed. The column was placed on a shaker platform and phosphitylation was allowed to occur for 2-4 hours. Next, a solution of 25-30 mg of ruthenium complex **6** in 150 μL acetonitrile was drawn into one syringe and 100 μL tetrazole activator solution was drawn into a second syringe. These were attached after washing the column briefly on the synthesizer by flushing with acetonitrile and reverse flushing with argon. The solutions were mixed and coupling was allowed to proceed overnight. Following overnight coupling, oxidation was performed on the synthesizer; capping was not performed since all of the hydroxyl groups on the complex were left unprotected and thus would result in synthesis termination. Synthesis of the remaining five DNA arms was performed on the synthesizer using either conventional 3'-CE phosphoramidites or reverse 5'-CE phosphoramidites[8] if conjugates with uniform sequence polarity were desired. The wait time for coupling of the first base after coupling of the complex was extended to 30 minute. This initial coupling was repeated three times. Wait times for all subsequent couplings were extended to 60 seconds to ensure complete extension of all arms.

Following deprotection in the conventional manner, conjugates were purified on a POROS 0.46cm x 10cm column packed with Oligo R3 reverse phase support with a flow rate of

5mL/min in 50mM triethylammonium acetate (pH 7.0) and an acetonitrile gradient (0% for 1 min followed by 0 to 70% over 11 minutes). A corresponding analytical trace of the mixture after deprotection is illustrated in Figure S5. After HPLC isolation, the terminal DMT groups were removed by reaction with 80% acetic acid for 30 min at 0^0C and samples were desalted on disposable Econo-pac G10 columns (Bio-Rad). Conjugates were further purified by denaturing PAGE (10%, 75:1 acrylamide:bisacrylamide). Bands corresponding to the desired conjugates were excised. The conjugates were then recovered by Elutrap, desalted, and lyophilized to dryness. The purity of the conjugates was confirmed by analysis by PAGE and by capillary electrophoresis.

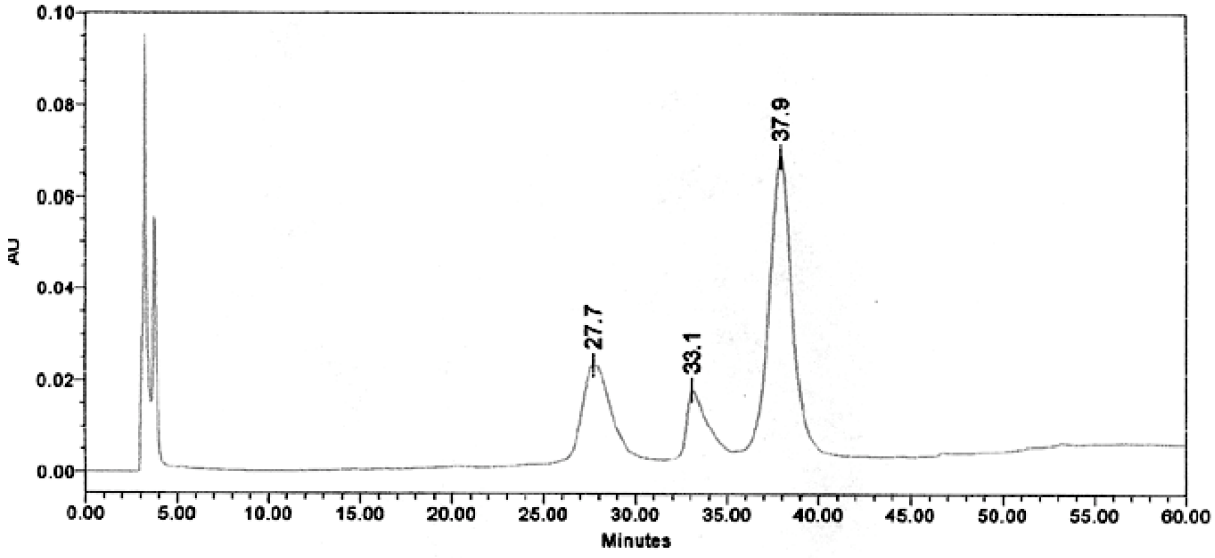


Figure S5. HPLC trace after deprotection of the Ru(II)DNA_6 assembly (terminal DMT groups remain), using an analytical C_{18} column in trimethyl ammonium acetate, pH 7.0 and a 0-70% gradient of acetonitrile over 60 min.

MALDI-TOF analyses

MALDI-TOF analyses were obtained for complexes **2** and **3**. With the high molecular weights of these complexes it was difficult to obtain peaks for specific masses. However, it was clear that we obtained mass ranges that corresponded to the complexes prepared. Figure S6

illustrates part of the analysis for complex **3**, which has a calculated mass of 38,022.7 and there are clearly peaks in that mass range for the M+ and as well as for the M^{2+} mass range.

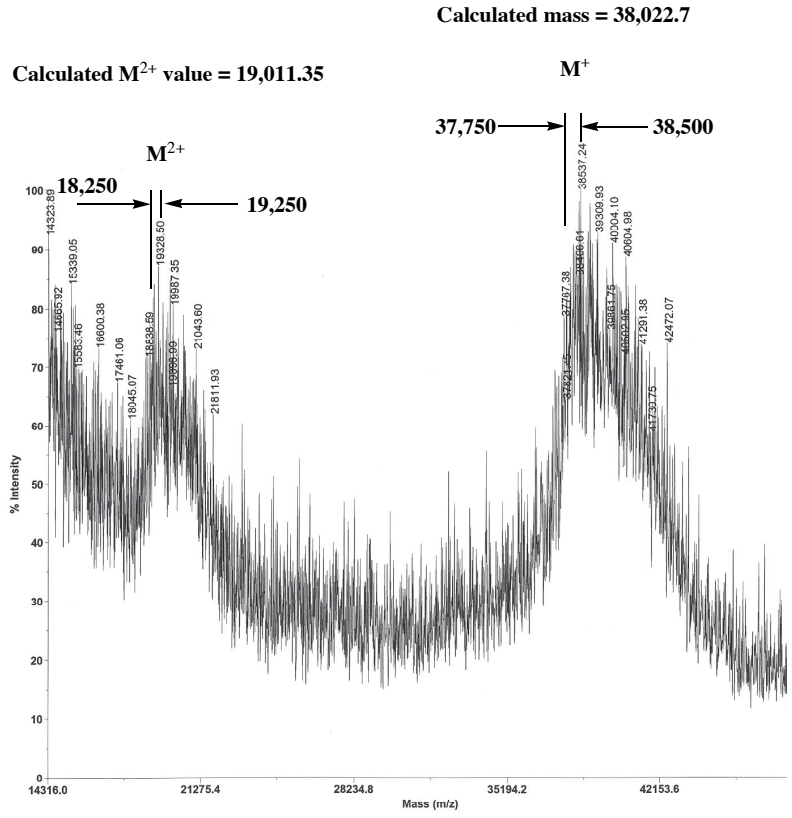


Figure S6. MALDI-TOF analysis of complex **3** with a calculated mass of 38,022.7.

UV-Vis Analyses UV-Vis analyses were performed to compare the absorbance ratios at 260 nm [DNA + Ru(II) tris(bipyridine)] and 460 nm [Ru(II) tris(bipyridine)]. An example of the spectra is illustrated in Figure S7.

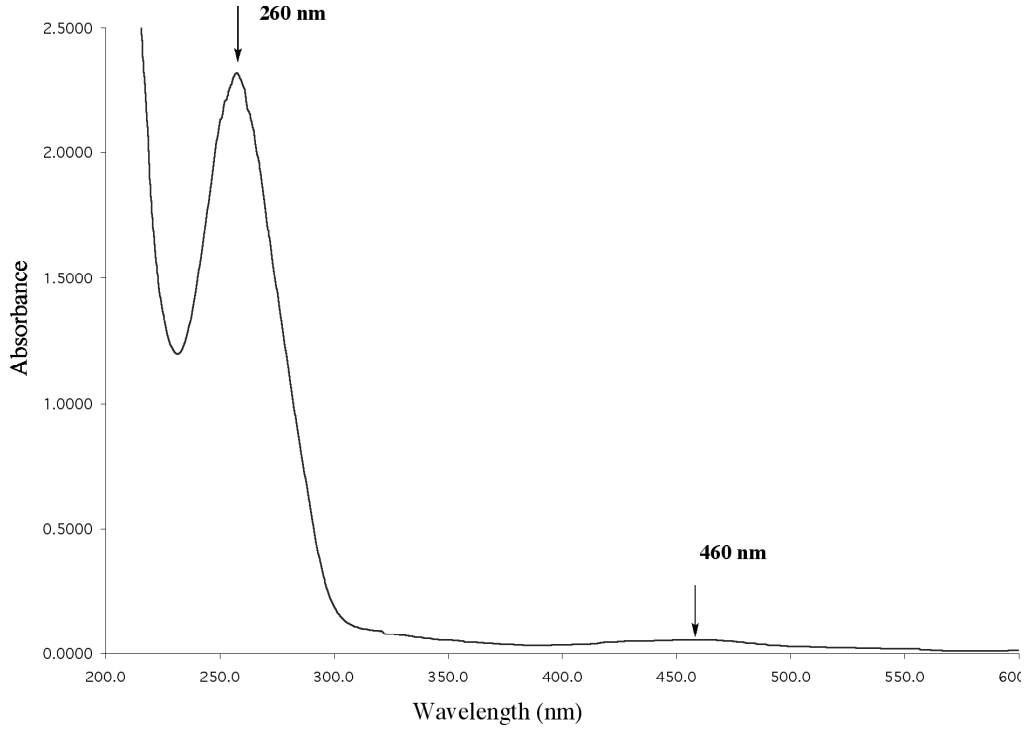


Figure S7. UV-Vis analysis of complex **2**.

UV-Vis Data

Parameter	Complex **2**	Complex **3**
ε_{260} (mM^{-1}) DNA 20-mer	197	181
ε_{260} (mM^{-1}) Ru(II) bipy3	19.6	19.6
ε_{460} (mM^{-1}) Ru(II) bipy3	15.9	15.9
A_{260} units	0.8629	0.693
A_{460} units	0.016	0.0086
[Ru(II) $bipy_3$] mM	6.3×10^{-4}	5.42×10^{-4}
A_{260} units for Ru(II) $bipby_3$	0.012	0.011
A_{260} units for DNA [Total A_{260} units – A_{260} units for Ru(II)]	0.86	0.63
[DNA] mM	4.36×10^{-3}	3.77×10^{-3}
[DNA]/[Ru(II) $bipy_3$]	**1.14**	**1.15**

The ratios of the concentration of DNA (120 nucleotides) to the concentration of Ru(II) $bipy_3$ are consistent, but generally slightly higher than the theoretical value of 1.0. This may reflect some hyperchromicity effects for the absorbance of the centrally located Ru(II) $bipy_3$ as well as systematic errors in measuring very low absorbance values (<0.02) at 460 nm. However, the values indicate that the DNA/Ru ratio is at least 1.0 and that all six of the DNA arms are present. Hybridization data confirms the presence of all six DNA arms (see below).

Hybridization experiments

For the hybridization studies, individual samples were prepared by adding the required amount of conjugate and complement to an eppendorf tube, followed by drying by speed vac. To the residue was added 5 μl 80 mM Tris-borate-1mM $MgCl_2$, pH 8.3. Samples were heated for 2 minutes at 95 ºC and allowed to cool gradually to ambient temperature. After cooling, 5μl of Ficoll loading buffer containing tris-borate-1mM $MgCl_2$ and tracking dyes was added to each sample. Samples were loaded on to 75:1 cross-linked 10% nondenaturing gels and were developed using a Tris-borate-1mM $MgCl_2$ buffer system at 150V for 8 to 10 hours. Larger complexes were analyzed by electrophoresis in 2.5% agarose gels developed in Tris-borate 1 mM $MgCl_2$. Hybridization events were visualized by ethidium bromide staining.

It is to be expected that after incorporation of the Ru(II) $bipy_3$ complex that the subsequent elongation of the remaining five linkers will not occur with high efficiency in all cases. We have observed that it is necessary to use 2000Å pore supports for the DNA syntheses. But even in this case, it can be expected that some of the linkers will not be in a position to react with phosphoramidites and be extended. To confirm one additional (the sixth) unhybridized that all six arms are present in the complexes studied, we have performed stepwise hybridization

studies with the complementary 20-mers. This analysis is illustrated in Figure 2 of the manuscript. In this figure the hybridization assay for two isolated complexes is illustrated. The two complexes exhibited different gel mobilities and the obvious interpretation was that one complex relative to the other had one or more additional DNA arms. The hybridization assay confirms that interpretation. In one case (left panel, Figure 2) six hybridization bands are observed during the stepwise addition of the complementary 20-mer. In the second case (right panel, Figure 2) only five hybridization products are present. Careful analysis of the gel also reveals that the fifth band on the left panel (representing five hybridized arms) migrates more slowly than does the fifth band on the right panel (also representing five hybridized arms). The differences in the mobilities arise from the fact that the complex in the left panel contains five hybridized arms plus a sixth single-stranded arm. The complex in the right panel contains only

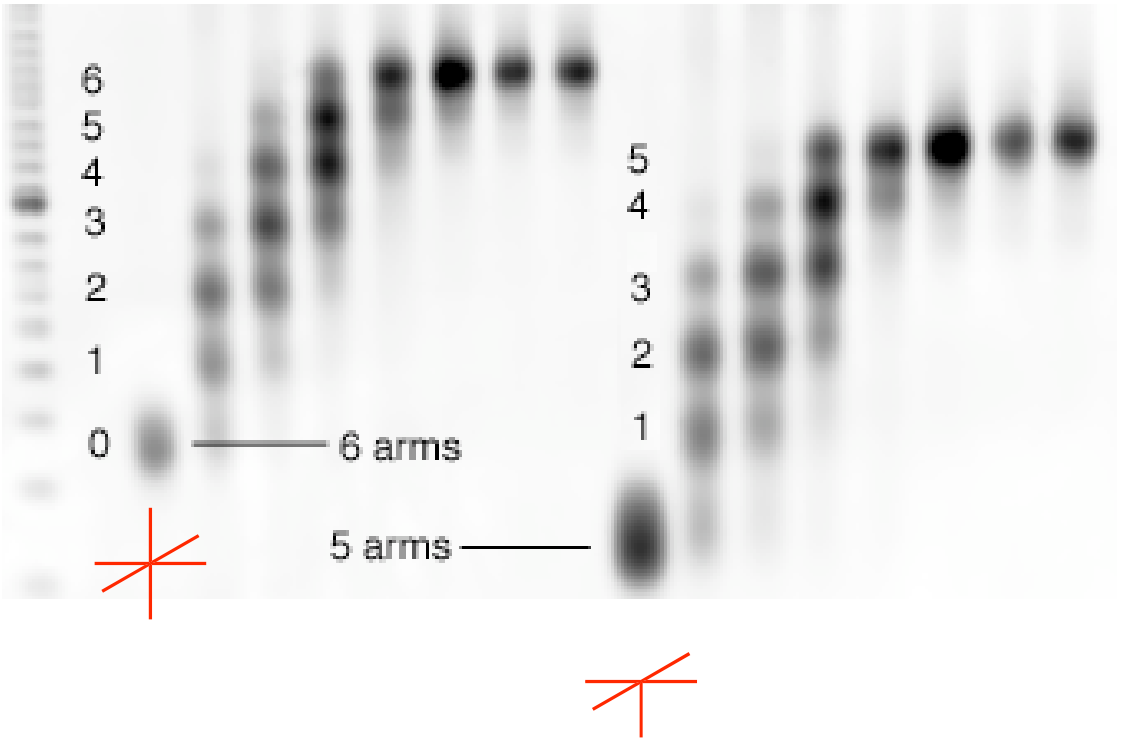


Figure 2 from manuscript – stepwise hybridization between the six- (and five)-arm complexes and the complementary 20-mer.

five hybridized DNA arms. As more complement is added to the sample in the right panel, no additional hybridization product appears. When the same is done to the complex of the left panel, the sixth hybridization band is present. These results indicate that the complex on the left contains all six DNA arms, whereas the complex on the right contains only 5 DNA arms.

Figure S8 illustrates a model of the relative positioning and spacing of the Ru-center and four of the six DNA arms. The fifth and sixth arms project into and out of the plane of the paper and have been eliminated for clarity.

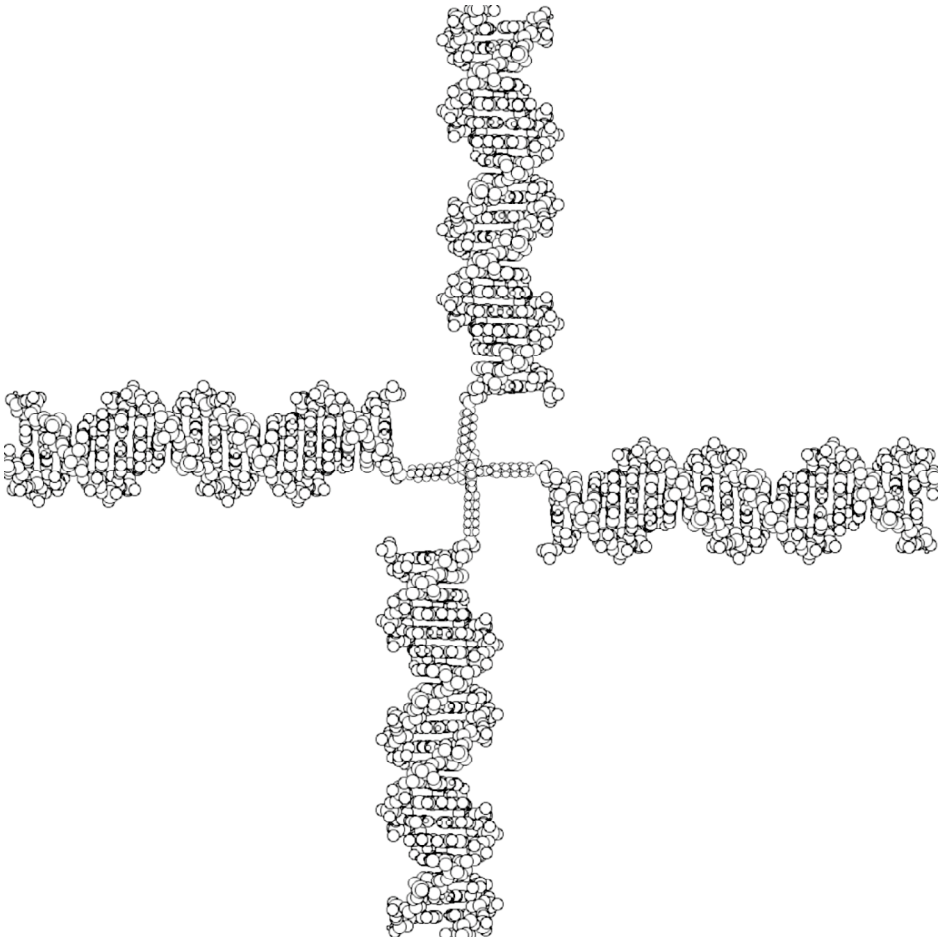


Figure S8. Model structure of a Ru(II)(bipy)$_3$ and nine carbon linkers tethering six DNA 20-mer duplexes. The two apical sequences have been removed for clarity.

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