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Interactions of cis-bis Ruthenium Complexes with Monosaccharides

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Interactions of cis-bis Ruthenium Complexes with Monosaccharides

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Lindsay is a senior Biology and Chemistry double major. This project began as volunteer work in Lindsay's sophomore year and has continued with the aid of several ATP grants throughout her undergraduate career. Lindsay and her mentor, Dr. Steven Haefner, have presented this work at the American Chemical Society conferences in San Diego and Atlanta, and will also present at the 2008 meeting in New Orleans. Lindsay has also presented this work at an international conference in Konstanz, Germany. This work is part of an honors thesis which will be defended in April of 2008. Lindsay plans to go on to medical school to pursue a joint MD/PhD degree.

Abstract

Molecular recognition and binding of oligosaccharides play an essential role in many biological processes including cell-cell recognition, signaling and adhesion. Our group is currently involved in the development of metal based receptors that can effectively bind specific glycoconjugates. Species that can selectively bind and chemically alter membrane glycoconjugates have the potential to inhibit tumor cell metastasis, inflammation and fibrosis. As an initial step towards our goal the interactions of cis-$Ru(bpy)_2(DMF)_2^{2+}$ (bpy = bipyridine, DMF = dimethylformamide) and cis-$Ru(acac)_2(CH_3CN)_2^{+}$ (acac = acetylacetonate) with the monosaccharides glucose and mannose have been examined to identify and understand the conditions necessary for complexation of sugars to ruthenium. Cis-$Ru(bpy)_2(DMF)_2^{2+}$ has been synthesized from cis-$Ru(bpy)_2Cl_2$ by addition of Ag^{+} which removes the chloride ions as silver chloride. This newly isolated compound represents a valuable precursor for binding studies with monosaccharides because we expect that the DMF ligands will be readily displaced. Binding studies with cis-$Ru(bpy)_2(DMF)_2^{2+}$ and simple monosaccharides indicate, however, that metal-sugar interactions are extremely weak. In order to strengthen these interactions, a second compound, cis-$Ru(bpy)_2(MeOH)_2^{2+}$ was isolated with the idea that the methanol group will be even more easily displaced than the DMF. Complexation studies suggest that addition of sugar to cis-$Ru(bpy)_2(MeOH)_2^{2+}$ resulted in modest changes in the electronic absorptions of cis-$Ru(bpy)_2(MeOH)_2^{2+}$. Such changes suggest that when weakly coordinating solvents such as methanol are present weak metal-saccharide complexation occurs in solution.

Introduction

Oligosaccharides in the form of glycolipids and glycoproteins line the surface of the cellular membrane and are vital for the molecular recognition and binding of proteins called lectins.[1] These oligosaccharide-lectin interactions are responsible for a variety of biological processes including cell-cell recognition, communication, regulation, and immune defense.[2,3,4] Expressed at the terminus of each membrane oligosaccharide are tetrasaccharide moieties called sialyl

1. St. Hilaire P.M.; Halkes K.M.; Crocker P.R.; Meldal M. *J. Comb. Chem.* **2003**, 5, 18-27.

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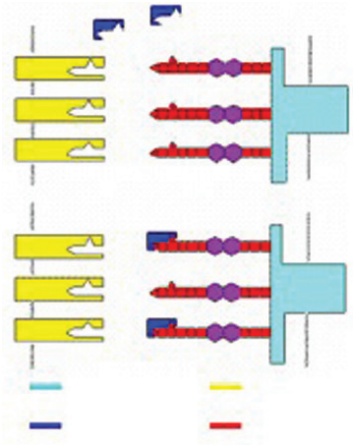
3. Dwek R.A. *Chem. Rev.* **1996**, 96, 683-720.

4. Dwek R.A. *Chem. Rev.* **2002**, 102, 283-284.

lewis^{x} and sialyl lewis^{a}.[5, 6] Sialyl groups are known to be over-expressed on many highly metastatic cancer cells including liver, pancreatic, breast, and lung carcinomas.[7] Accordingly, it is believed that the interactions of lectins with sialyl lewis^{x} and sialyl lewis^{a} play a considerable role in cancer cell metastasis. This evidence suggests that a small molecule capable of inhibiting the binding of lectins to the sialyl groups may slow metastasis by inhibiting adhesion of cancerous cells to local endothelium; ultimately preventing the initial step in the spreading of malignant tumor cells. Currently there are two strategies for the inhibition of carbohydrate lectin interactions. The most common approach has been to develop compounds that mimic the sialyl groups that are naturally expressed, causing the lectins to target the mimetics rather than the endothelial cells.[8, 9]

A second approach, the long-term goal of this project, is to develop metal based artificial receptors that selectively bind and essentially cap the sialyl groups of membrane glycoconjugates (Figure 1). This "capping" action will thereby prevent the recognition of the saccharides by the lectins, and potentially inhibit adhesion. To inhibit such carbohydrate-lectin interactions the associations between the molecules must be stronger than the attractive forces that exist between lectins and sialyl groups. Lectins primarily interact with sialyl groups through relatively weak multi-valent hydrogen bonding. A receptor that is capable of bonding covalently with multiple hydroxyl groups of the sugar therefore would be far more competitive. The long-term goal of developing such receptors is predicated on the hypothesis that the metal-oxygen covalent bond will be inherently stronger than the hydrogen bonds of the lectin.

Figure 1.



Metal ions, including magnesium and calcium, have been found to participate in the mediation of carbohydrate recognition in several metal based lectins including conconavalin and isolectin.[10] The presence of metals in carbohydrate based receptors is not surprising in that each of the hydroxyl groups found on carbohydrates represent binding sites in the ideal configuration for forming metal chelates. Despite the capacity for metal binding, this area of carbohydrate chemistry remains relatively unexplored.[11, 12] To date, only a limited number of well-characterized metal-carbohydrate complexes have been reported, particularly amongst the second and third row transition metals.[13, 14, 15, 16] In an attempt to expand this field we are examining the reactivity of cis-bis chelate complexes of the general type Ru(L-L)$_2$(solv)$_2$ with simple monosaccharides. The use of cis-bis metal complexes for carbohydrate coordination is predicated on the idea that chelating ligands such as bipyridine and acetylacetonate will readily retain their cis geometry around the metal center leaving two available sites for saccharide complexation. Furthermore, chelating ligands will prevent the formation of coordination polymers between the metal ion and the sugars. The remaining coordination sites of the metal center are occupied by labile solvent molecules such as dimethylformamide or acetonitrile which may be readily displaced by the hydroxyl groups of the sugar. With this in mind, we have synthesized the partially solvated Ru(II) complex cis-[Ru(bpy)$_2$(DMF)$_2$][BF$_4$]$_2$ (**1**) and cis-[Ru(bpy)$_2$(CH$_3$OH)$_2$][BF$_4$]$_2$ (**2**), as well as the tetrafluoroborate salt of cis-[Ru(acac)$_2$(CH$_3$CN)$_2$]$^+$ (**3**). Herein we report the reactivity of compounds **1-3** with glucose and mannose.

Results and Discussion

Synthesis and Characterization of solvated cis-bis-Ruthenium Complexes.

Cis-[Ru(bpy)$_2$(DMF)$_2$][BF$_4$]$_2$ (**1**) was chosen as an initial molecule of interest because the cis geometry and chelating nature of the bipyridine ligands retains available cis-coordination sites in the appropriate configuration for metal-saccharide complexation. These sites are temporarily maintained by the fairly labile DMF ligand that can be readily displaced by a more favorable, or more strongly complexing molecule. Cis-[Ru(bpy)$_2$(DMF)$_2$][BF$_4$]$_2$ (**1**) has been synthesized from ruthenium dichloride in DMF by addition of silver tetrafluoroborate. The addition of Ag$^+$ ion causes the coordinated chloride ions to precipitate as silver chloride. Because of the non-coordinating nature of the BF$_4^-$ counterion, the chloride ions are replaced by the DMF solvent. The red-violet

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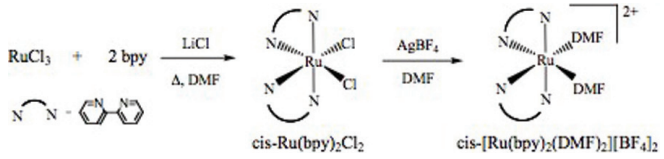
16. Steinborn D.; Junicke H. *Chem. Rev.* **2000**, 100, 4283-4317.

solution thus produced is filtered to remove the precipitated solid. Despite the expected inertness of a low spin d^6 metal ion, the loss of chloride ion occurs rather quickly, only requiring several hours of stirring at room temperature (Equation 1). The compound can be isolated cleanly in 61% yield by stripping away the solvent under vacuum and washing the resulting residue with hexanes and tetrahydrofuran. Alternatively, single crystals suitable for x-ray diffraction studies may be obtained by slow diffusion of tetrahydrofuran through a layer of hexanes into a DMF solution of the compound. The compound in situ is air stable, however the isolated compound is tends to be hygroscopic and is best stored in a dry environment.

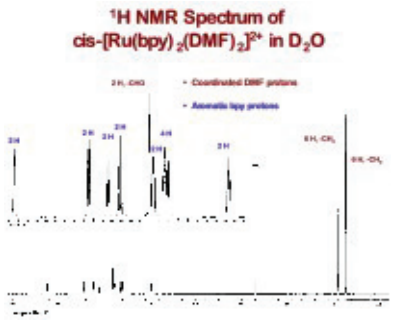
Equation 1.



While generally unremarkable the IR spectrum of **1** reveals the presence of BF_4^- counterion ((νB-F) = 1064 cm^{-1}) corresponding to the removal of chloride ions. Additionally, the IR shows stretching vibrations for coordinated DMF ligands ((νC=O) = 1645 cm^{-1}). The shift of the carbonyl band to lower energy compared to free DMF is consistent with an oxygen bound DMF ligand. The ^1H NMR spectrum in D_2O shows eight resonances in the aromatic region ranging from 7.13 to 9.29 ppm (Figure 2). These resonances correspond to the two magnetically inequivalent rings of the bipyridine ligand; where one ring is trans to the DMF ligand and the second ring is adjacent to the DMF. This inequivalency is characteristic of bipyridines in a cis disposition. Resonances for the two coordinated DMF ligands appear at 2.73, 2.89 and 7.80 ppm. The electronic spectrum in DMF exhibits broad absorptions at 357 nm and 507 nm. Both of these absorbances are extremely intense giving dark, red-violet colored solutions. Consequently, samples must be made extremely dilute in order to obtain accurate and on-scale spectra.

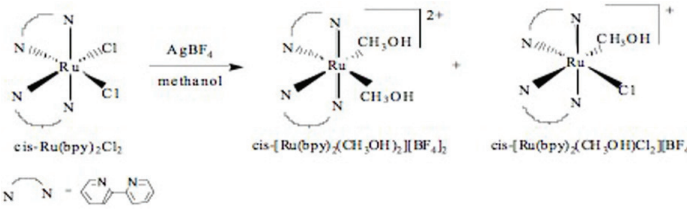
In an attempt to increase the affinity for saccharide coordination we have also prepared and attempted to isolate the analogous methanol complex, cis-$[Ru(bpy)_2(CH_3OH)_2][BF_4]_2$. The coordinated methanol ligands should be even more weakly held than the DMF ligands, thus increasing the favorability for complexation to monosaccharides. The preparation for this compound has been reported previously by Rillema,[17] but the compound was not isolated from the reaction mixture. Addition of methanol to a mixture of cis-$Ru(bpy)_2Cl_2{\cdot}2H_2O$ and two molar equivalents of $AgBF_4$ produces a red-orange solution after stirring for 12 hours. After filtration to remove the AgCl byproduct, a red solid was obtained by subsequent evaporation of the solvent. A ^1H NMR spectrum of the isolated solid in D_2O revealed the presence of a second ruthenium bipyridine complex as a minor impurity.

Figure 2



The spectrum of the major product exhibits a total of eight resonances in the aromatic region typical of a cis, disubstituted bipyridine complex. The minor product, estimated by integration to comprise 20% of the mixture, shows sixteen resonances. This large number of aromatic signals indicates a highly assymmetric structure and strongly suggests the presence of a monosubstituted species (Equation 2). Nevertheless, this sample was used in-situ to perform a qualitative titration with glucose (*vide infra*).

Equation 2.



A third compound, cis-$[Ru(acac)_2(CH_3CN)_2][BF_4]$ (**3**), was also prepared. This compound was selected because of the cis orientation of labile ligands which is similar in characteristic to the bipyridine compound. Cis-$Ru(acac)_2(CH_3CN)_2^+$, however, should have a higher affinity for saccharide coordination due to the replacement of the nitrogen donating atoms of the bipyridine for the oxygen donating acetylacetonate ligands. The harder oxygen donors should electronically tune the metal center for coordination to a second oxygen donating molecule, in this instance the hydroxyl groups of the monosaccharide. Furthermore, the higher oxidation state, (Ru(III) in **3** vs. Ru(II) in **1**), should enhance sugar coordination.

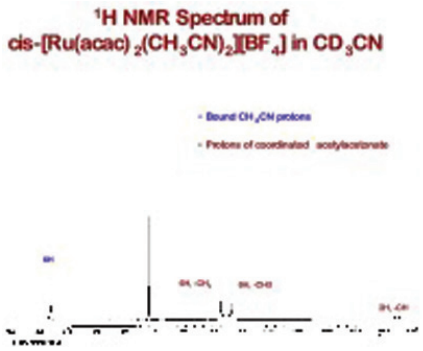
Cis-$[Ru(acac)_2(CH_3CN)_2][BF_4]$ was prepared based on a modification of the procedure first reported by Shimizu.[18]

17. Rillema, D.P; Macatangay, A; Zheng, G.Y; Jackman, D.C; Merkert, J.W. *Inorg. Chem.* **1996**, 35, 6823- 6831.

18. Kasahara, Y; Hoshino, Y; Shimizu K; Sato G.P. *Chem. Lett.* **1990**, 381-384.

Ruthenium(III) trisacetylacetonate ($Ru(acac)_3$) was stirred in acetonitirile with HBF_4 resulting in a color change from magenta to purple. This is indicative of the removal of one acetylacetonate ligand and exchange for two acetonitrile ligands. Removal of the solvent, followed by dissolution in water and subsequent extraction with an acetonitrile-methylene chloride mixture, results in a purple-blue solution from which **3** is obtained in 90% yield. The complex is soluble in polar solvents such as water, dimethylformamide, and dimethylacetamide. It also undergoes slow solvent ligand exchange as monitored by Uv-vis spectroscopy. The solvent exchange process is kinetically slow, but full exchange does occur in 72+ hours with most solvents.

Figure 3



The IR spectrum of cis-$Ru(acac)_2(CH_3CN)_2][BF_4]$ shows the presence of the BF_4^- anion, as well as the presence of acetonitrile indicated by the CN stretch at 2296 cm^{-1}. The ^1H NMR spectrum in D_2O reveals four major resonances which is consistent with the reported spectral data by Shimizu[18] (Figure 3). Two major signals are seen for the coordinated acetylacetonate protons at -23 and -27 ppm, as well as a resonance for the methine protons at -85 ppm. The additional signal is for the coordinated acetonitrile protons and appears at 36 ppm. Each resonance is broadened and shifted, consistent with the paramagenetic nature of the Ru(III) complex.

Reactivity with monosaccharides. Simple addition of excess glucose or mannose to $Ru(bpy)_2(DMF)_2^{2+}$ (**1**) in either DMF or H_2O at room temperature produced no dramatic changes in the UV-vis spectrum. Monitoring the reaction by ^1H NMR also shows no significant changes. Likewise, $Ru(acac)_2(CH_3CN)_2^+$ showed no obvious spectral evidence of complexation in H_2O. Taken together, these results suggest that sugar coordination is extremely weak, if at all existent. In both instances the hydroxyl groups are unable to compete with the coordinating solvent which is present in a substantial excess in solution.

Klufers[7] and Striegler[19] have shown that saccharide complexation may be enhanced by the addition of a base to deprotonate the hydroxyl groups of the sugars thereby creating a better ligand. This information led us to examine the effects that the addition of various bases would have on the cis-bis chelates of ruthenium. Addition of sodium hydroxide to aqueous solutions of **1** and **3** caused an immediate color change resulting from the coordination of hydroxide ion to the metal center. Addition of increasing amounts of glucose to **1** and **3** in strongly basic aqueous solution resulted in no discernable change in their electronic spectra (Figure 4-6). The non-shifting ruthenium absorptions indicate that the hydroxyl groups of the saccharide are unable to adequately compete with the hydroxide ion for metal complexation even in the presence of excess sugar. A similar study was also performed with **1** and **3** using sodium bicarbonate as a base. Reaction of the bicarbonate ion HCO_3^- with acidic protons would produce CO_2 which would then be released as a gas. Addition of $NaHCO_3$ immediately produced a color change in both **1** and **3**. However, addition of excess glucose failed to produce any further changes in the subsequent UV-vis spectra.

Together, these studies lead to the conclusion that hydroxide is too strongly coordinating and therefore must be avoided. Fully eliminating hydroxide ion requires discontinuing the use of aqueous solutions and replacing them with a weakly coordinating solvent. The solvent must be less coordinating than DMF, yet still dissolve the sugar. To this end, we have synthesized the methanol species cis-$[Ru(bpy)_2(CH_3OH)_2][BF_4]_2$ (*vide supra*) and investigated its reactivity with glucose. The methanol solvated species in solution exhibits increasing absorption maxima upon addition of glucose in a qualitative titration. The band at 500 nm shows a slight red shift and the band at 350 nm blue shifts with increasing monosaccharide concentration (Figure 7). Changes of this nature, coupled with the increasing absorption maxima suggest that binding is occurring in solution. Not surprisingly, these binding associations are weak. ^1H NMR suggests that the sugar is dynamically exchanging with the free sugar present in solution. In order to further strengthen complexation, it may be necessary to remove the hydroxyl protons of the sugar by the addition of a non-coordinating base, such as 1,8-bis-(dimethylamino)-napthalene.

Conclusion. We have been able to prepare solvated cis-bis-chelate complexes of ruthenium by halide abstraction from $Ru(bpy)_2Cl_2$. Based upon their ^1H NMR spectra, both species maintain their cis coordinating geometries. Complexation studies by UV-vis and ^1H NMR with monosaccharides suggest that the use of a weakly coordinating solvent in non aqueous solutions greatly enhances metal-sugar binding. We anticipate that the addition of a non-coordinating base will improve coordination. Future studies

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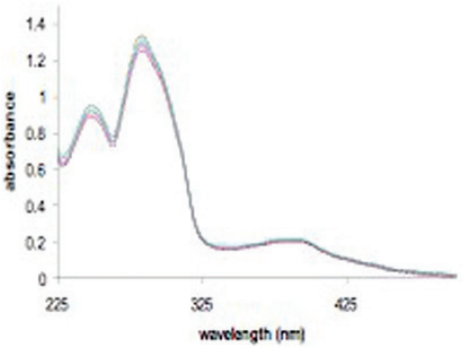

Figure 4. Titration of 1 with glucose from (0-15) equivalents.

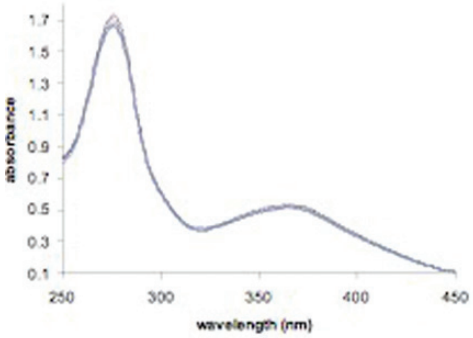

Figure 5. Titration of 3 with glucose from (0-15) equivalents.

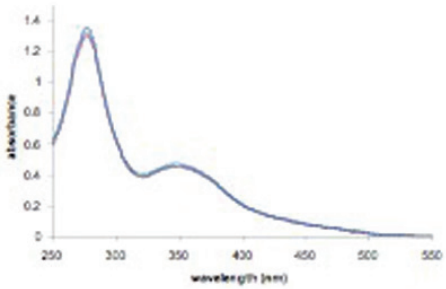

Figure 6. Titration of 3 with mannose from (0-15) equivalents .

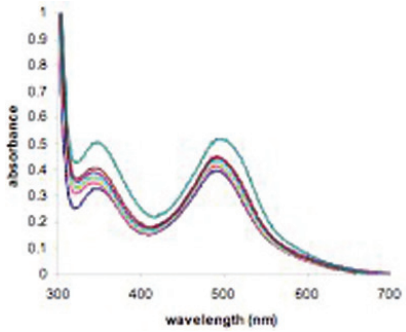

Figure 7. Qualitative titration of 2 with glucose in methanol.

will focus on identification of the exact conditions necessary to strengthen metal-saccharide complexation. This information, in turn, will lead to the isolation of strongly complexed species, as well as development of more sophisticated bi- and poly-metallic species for the selective binding of oligosaccharides.

Experimental

General Considerations. All manipulations were performed in air except where noted. Air and moisture sensitive compounds were manipulated using standard Schlenk techniques or in a dry box. All solvents and reagents were purchased from commercial sources and used as received. Silver tetrafluoroborate was purchased from Aldrich and ruthenium(III) trisacetylacetonate was purchased from STREM. Uv-vis spectra were collected on a Hewlett-Packard 8543 diode-array spectrophotometer. Infrared spectroscopies were performed as a Nujol mull between sodium chloride plates. ^{1}H NMR spectroscopies were performed on either a 400 or 600 MHz JEOL-ECX spectrometer.

Preparations.

cis-Ru(bpy)$_2$Cl$_2$·2H$_2$O. The compound was prepared according to a modification of techniques previously published.[20] A mixture of 3.9 g RuCl$_3$·xH$_2$O (0.0149 moles), 4.68 g bipyridine (0.03 moles) and 2.9 g LiCl (0.0684 mol) were refluxed under argon in dimethylformamide (25 mL) for 8 hours. During this time a series of color changes were observed that ultimately gave a dark plum colored solution. After cooling to room temperature, 125 mL of acetone was added and the resulting suspension was filtered through a medium porosity frit in open air. The dark green-black microcrystalline product was washed with 3 x 25 mL portions of distilled water, followed by 3 x 25 mL diethyl ether. An additional 5 mL of distilled water was used to remove residual product from flask along with 10 mL additional diethyl ether. The product was then dried by vacuum, 5.55 g. (71% yield based on ruthenium).

cis-[Ru(bpy)$_2$(DMF)$_2$][BF$_4$]$_2$ (1). To a 20 mL vial, 0.100 g (0.192 mmoles) Ru(bpy)$_2$Cl$_2$·2H$_2$O, and 0.075 g (0.385 mmoles) AgBF$_4$ was added, and the mixture was stirred in 3 mL dimethylformamide overnight. A small amount of celite was used in a medium porosity frit to aid filtration of the AgCl precipitate from the red-violet solution. The celite plug was washed with an additional 2 mL dimethylformamide. The solvent was then removed by rotary evaporation. The resulting garnet colored residue was washed with hexanes and tetrahydrofuran and dried under vacuum. 0.086 g (61% yield based on ruthenium). UV(dmf) λ_{max}, nm 357, 503. IR (Nujol, NaCl) C=O 1645 cm^{-1}, B-F 1063 cm^{-1}. ^{1}H NMR (D$_2$O, δ): 9.29 (d, 2H), 8.65 (d, 2H), 8.43 (d, 2H), 8.23 (t, 2H), 7.89 (s, 2H), 7.75 (t, 2H), 7.57 (m, 4H), 7.13 (t, 2H), 2.89 (s, 6H), 2.73 (s, 6H).

20. Sullivan B.P.; Salmon D.J.; Meyer, T.J. *Inorg. Chem.* **1978**, 17, 3334-3341.

cis-[Ru(bpy)$_2$(CH$_3$OH)$_2$][BF$_4$]$_2$ (2). In a 20 mL vial, 0.100 g (0.192 mmoles) Ru(bpy)$_2$Cl$_2$·2H$_2$O, and 0.075 g (0.385 mmoles) AgBF$_4$ were added to 3 mL methanol. After stirring overnight, the solution was filtered to remove AgCl(s) and washed with an additional 2 mL of methanol. Rotary evaporation was used to dry the red-orange solution. The reside was washed with hexanes and tetrahydrofuran yielding a red microcrystalline solid. 0.083 g (68% yield based on ruthenium). UV (methanol) λ_{max}, nm 348, 492. ^{1}H NMR (D$_2$O, δ): 9.24 (d, 1H), 8.45 (d, 1H), 8.24 (d, 1H), 8.12 (t, 1H), 7.78 (m, 2H), 7.62 (m, 2H), 6.97 (t, 1H).

cis-[Ru(acac)$_2$(CH$_3$CN)$_2$][BF$_4$] (3). In a 200 mL round bottom flask, 0.500 g (1.36 mmoles) Ru(acac)$_3$ was added to 100 mL acetonitrile. 0.2 mL HBF$_4$ was added resulting in an immediate, gradually intensifying color change from magenta to purple. The solution was stirred for one hour after which the solvent was removed by rotary evaporation. The purple-blue residue was washed three times with hexanes. The residue was then redissolved in 40 mL of water and the compound was extracted with a 7:3 mixture of methylene chloride and acetonitrile solution which was added in 100 mL portions. The solution was then stirred over magnesium sulfate for 30 minutes, filtered, and washed with additional methylene chloride. The solvent was then removed by rotary evaporation and the solid dried under vacuum. (90% yield based on ruthenium). UV $\lambda_{max,}$ nm 283, 580. IR (Nujol, NaCl) CN 2296 cm^{-1}, B-F 1058 cm^{-1}. ^{1}H NMR (D$_2$O, δ): -85 (s 2H), -27 (s, 6H), -23 (s, 6H), 36 (s, 6H).

Titrations with monosaccharides. All titrations were monitored by Uv-vis spectroscopy and were performed using quartz cuvettes with a 1 cm pathlength over a range of 200-900 nm. Titrations were performed at room temperature in 0.01 M NaOH(aq) solution. 0.01 M saccharide solution was added in increasing amounts to 0.085 mM Ru(acac)$_2$(CH$_3$CN)$_2^+$ or 0.055 mM Ru(bpy)$_2$(DMF)$_2^{2+}$ solutions. The total volume and total concentration of metal complex were kept constant at 2 ml throughout the duration of the experiment by adding the appropriate amount of 0.01M sodium hydroxide solution. In each instance dissolution of the metal complexes produces an immediate color change, presumably due to the displacement of the weakly held solvent by hydroxide ions. With the exception of the stock solutions the Uv-vis absorbances of the resulting mixtures were measured immediately upon preparation.

Acknowledgement
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