8-5-2015

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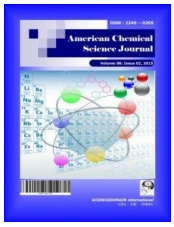

American Chemical Science Journal
9(1): 1-7, 2015, Article no.ACSJ.19681
ISSN: 2249-0205

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Polycations XXII. $Ru(bpy)_2L_2$ and $Ru(phen)_2L_2$ Systems with Cationic 4,4'-Bipyridine Ligands: Syntheses, Characteristics, and Interactions with DNA

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Authors' contributions

This work was performed with collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACSJ/2015/19681
Editor(s):
(1) Mohammad Luqman, Department of Basic Science, College of Applied Sciences, A'Sharqiyah University, Oman.
Reviewers:
(1) Sandeep Rai, Shroff S. R. Rotary Institute of Chemical Technology, Gujarat, India.
(2) Dolunay Sakar Dasdan, Yildiz Technical University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/10410

Original Research Article

Received 23rd June 2015
Accepted 19th July 2015
Published 5th August 2015

ABSTRACT

Earlier efforts have been concerned with the association of Ruthenium octahedral complexes with DNA. Since the positively charged ruthenium species has been found to associate with the electron rich major groove of double-stranded DNA, it was proposed the addition of cationic sites on the ligands attached to ruthenium would facilitate such association. Thus, we have synthesized several series of octahedral ruthenium complexes bearing ligands having within themselves cationic sites. These have been investigated in their interaction with calf thymus DNA using fluorescence titration analysis. The introduction of the cationic ligands has been found to exhibit enhanced association of the ruthenium (II) species with the DNA as compared to those without such ligands, in keeping with the original concept. This work leads us to conclude that this line of investigation can lead to useful pharmaceutical agents.

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Keywords: Polycations; DNA-binding; bipyridine; quaternary ammonium; ruthenium (II) complex.

1. INTRODUCTION

1.1 Prior Efforts from our Laboratory

Following on our work concerning the interactions of polycationic organic salts with DNA [1] and our preparation of several series of polycationic derivatives of Ru(II) complexes, [2] we have been concerned with the preparation of a series of compounds of the general category $Ru(bpy)_2L_2(X^-)_n$ [and. to a lesser extent, $Ru(phen)_2L_2(X^-)_m$] wherein the ligands L bear additional cationic sites. As it is known [3] that systems of the general type $Ru(bpy)_2L_2(X^-)_m$ exhibit interactions with DNA and are of interest as potential antitumor agents through these interactions, it was postulated that the addition of cationic sites within the ligands L would enhance their ability to interact with DNA (through binding to the anionic major groove of double stranded DNA) and potentially increase the potency of the agent.

1.2 Concept of the Current Work

The current report is concerned particularly with those systems wherein L is derived from the parent 4,4'-bipyridyl unit with one of the nitrogen sites being quaternized with the addition of an alkyl group (See Fig. 1).

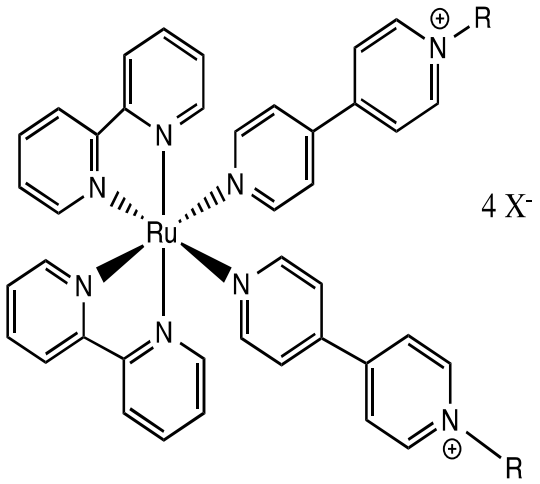


Fig. 1. General structure of the bis(2,2'-bipyridyl) octahedral ruthenium(II) species under consideration in the current report. Additional compounds of interest have (phen) bidentate ligands in place of the bipyridyl (bpy) ligands

Through the investigation of models of the DNA and the complexes, it is anticipated that such structures would be able to associate with double stranded DNA using both the ruthenium(II) site and the distal charged quaternary ammonium sites within the two ligands L, the latter being electrostatically associated with the major groove of the DNA. In addition to their syntheses, the electrochemical characteristics of these species has been measured along with their optical characteristics and notation of their binding characteristics with double stranded DNA.

The current investigation involves measurement of the binding of the ruthenium(II) species with double-stranded DNA and a comparison of the binding constants for these new complexes with ruthenium(II) species in which "ordinary" (with no pendant charged sites) ligands are present.

2. EXPERIMENTAL

2.1 General

All materials used in preparations were reagent quality of commercial origin and used without further purification unless otherwise noted. All ^1H and ^{13}C NMR spectra (including dept 45 ^{13}C spectra for determination of carbon sites devoid of attached H, such signals noted by "q") were measured in commercial deuterated solvents using a Brüker 400 MHz DPX400 instrument. Elemental analyses were performed by Columbia Analytical Services of Tucson, AZ. UV/Vis spectra were measured using anAgilent 8453 spectrometer. Fluorescence measurements were performed using a Horiba Jobin Yvon FluoroMax-P spectrometer interfaced with FluorEssence software. Cyclic voltammetry determinations were performed using an eDAQ 466 potentiostat instrument system, with acetonitrile solutions having tetraethylammonium tetrafluoroborate (10^{-1} *M*) as the electrolyte, measured from -2.0 to +2.0 V at 50 mV/sec.

2.2 Synthetic Procedures

2.2.1 General procedure for the preparation of 1-substituted-[4,4'-bipyridin]-1-ium halides

The appropriate haloalkane (0.05 mol) was taken with one equivalent amount (0.05 mol) of 4,4'-bipyridine in 250 mL of ethyl acetate and heated at reflux for 20 hr. After this time the reaction mixture was cooled and the solid adduct was

isolated by suction filtration through sintered glass, washed with additional ethyl acetate (100 mL) and dried under vacuum. No further purification was required. NMR spectra (^{1}H and ^{13}C) were measured in D_2O solution to verify structure and UV/Vis spectra and cyclic voltammograms were measured using standard methods. Data (yield, UV/Vis absorptions, analyses, and NMR spectra) are presented in Table 1 for the newly synthesized materials.

2.2.2 General procedure for the preparation of cis-bis-(2,2'-bipyridine)-cis-bis-(1-substituted-[4,4'-bipyridin]-1-ium)ruthenium tetrahalides

The appropriate 1-substituted-[4,4'-bipyridin]-1-ium halide (0.004 mol) was taken with one-half molar amount (0.002 mol) of *cis*-bis(-2,2'-bipyridine) dichlororuthenium(II) dihydrate in 50 mL 95% ethanol and heated at reflux for 18 hr. After filtering through sintered glass, the solution was evaporated under reduced pressure to provide the trarget material which required no further purification. NMR spectra (^{1}H and ^{13}C) were measured in D_2O solution to verify structure and UV/Vis spectra and cyclic voltammograms were measured using standard methods. Data (yield, UV/Vis absorptions, analyses, and NMR spectra) are presented in Table 2 for the newly synthesized materials.

2.3 Cyclic Voltammetry Scans

All cyclic voltammetry scans were made using an eDAQ 466 potentiostat with a glassy carbon working electrode, Ag/AgCl reference electrode, and a Pt/Ti wire anode. The electrolyte was 0.1 *M* tetraethylammonium tetrafluoroborate in acetonitrile, using the analyte at ~3 *mM* concentration. Scans over the range of -2.0 V to +2.0 V were made at 50 mV/sec.

2.4 DNA Binding Investigations Using Fluorescence Titration Analysis

The indicated ruthenium complexes were investigated by using solutions containing the specific ruthenium complex and an excess of calf thymus DNA to titrate a solution containing only the ruthenium complex at the same concentration as present in the DNA-containing solution. Excitation was performed using 475 nm light and subsequent fluorescence intensity was noted and used to calculate binding constants for the complexes bearing particular ligands using a modified Eadie-Hofstee method.

3. RESULTS AND DISCUSSION

3.1 Syntheses

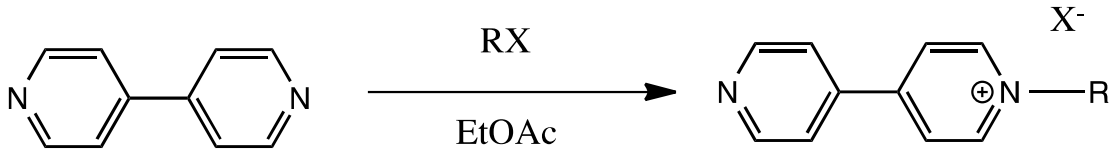
The cationic ligands for the present investigation have been prepared using a standard procedure for reaction of the diamine, 4,4'-dipyridyl, with a single equivalent amount of the appropriate haloalkane in ethyl acetate solution, as shown in Scheme 1. As has been noted in prior investigations, the use of ethyl acetate as a medium for the quaternization facilitates immediate precipitation of the mono-alkylated species, thereby preventing additional alkylation. [4] Syntheses, spectrophotometric, and analytical data for the newly synthesized cationic ligands are exhibited in Table 1. All materials prepared are mildly hydroscopic but can be rendered anhydrous readily under high vacuum at ambient temperature. All are virtually colorless and exhibit optical properties as anticipated for simple quaternary ammonium halide salts. These UV/Vis spectral characteristics are noted in Table 1.

Subsequent to the preparation of the ligands of interest, complexation with ruthenium(II) is accomplished using a standard procedure [5] that provides the target complexes with the cationic ligands in very good yield. The syntheses, spectrophotometric, and analytical data for the newly synthesized complexes are exhibited in Table 2. All materials are moderately hydroscopic but can be rendered anhydrous readily for analytical purposes using high vacuum at ambient temperature. After exposure to moist air, all of them congeal from a powdery state to a brittle solid before becoming liquid, but are readily converted back to an anhydrous condition under vacuum. All exhibit optical properties consistent with octahedral ruthenium(II) complexes.

3.2 Cyclic Voltammetry

Cyclic voltammetry determinations were made for all of the newly prepared compounds in order to ascertain oxidation/reduction characteristics of both the ligands and the ruthenium(II) complexes. Intriguingly, the cationic ligands (1)-(9) all exhibit an irreversible oxidation at +0.60 to +1.35 V. While simple tetraalkylammonium salts are neither oxidized nor reduced under standard

cyclic voltammetry conditions, these materials exhibit several such processes, one oxidation being irreversible. A typical example is shown in Fig. 2 for (4) wherein three reversible redox processes are also noted, an observation typical of all of the 1-substituted-[4,4'bipyridin]-1-ium halides. (The *m*-methoxybenzyl-substituted compound (8) is atypical (compared to other salts investigated herein) in that it exhibits an additional reversible redox process at -1.80 V.).



R = (**1**) CH_2CHCH_2
(**2**) $CH_2C(CH_3)CH_2$
(**3**) $CH_2CHC(CH_3)_2$
(**4**) C_6H_{13}
(**5**) $CH_2C_6H_5$
(**6**) CH_2-*p*-$C_6H_4NO_2$
(**7**) CH_2-*p*-$C_6H_4OCH_3$
(**8**) CH_2-*m*-$C_6H_4OCH_3$
(**9**) CH_2-*p*-C_6H_4Br

Scheme 1. Preparation of 1-substituted-[4,4'-bipyridin]-1-ium halides - Product details shown in Table 1

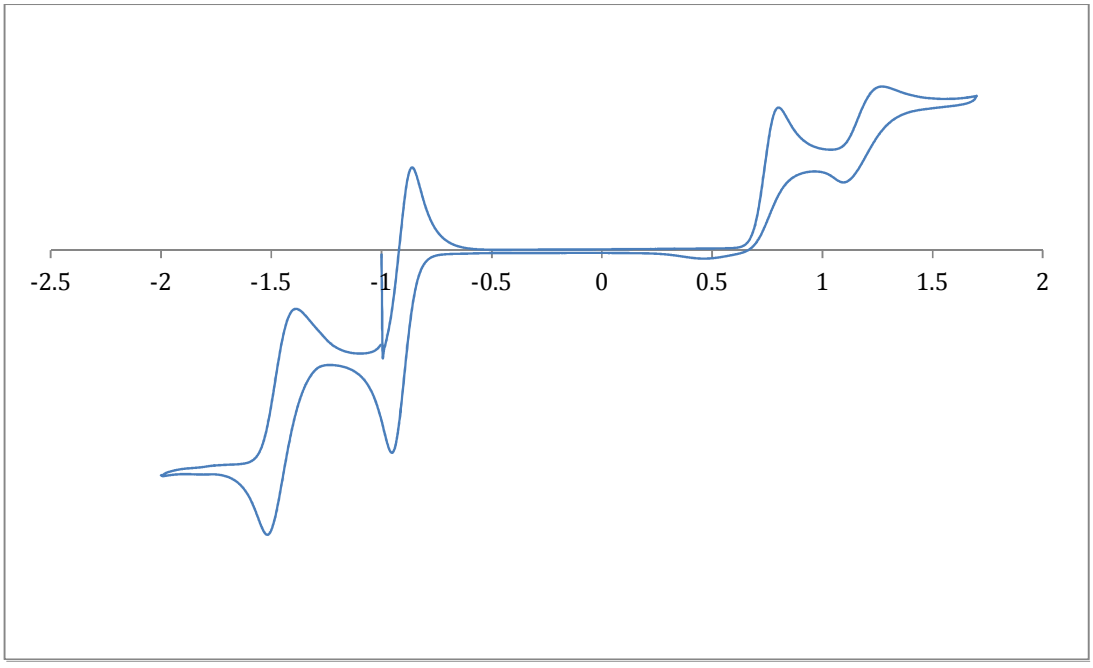


Fig. 2. Cyclic voltammetry scan for (4) - Irreversible oxidation, here at +0.84 V, is typical of the 1-substituted-[4,4'-bipyridin]-1-ium halides, the value varying with the structure of the substituent. The reversible redox processes noted are also typical of these materials, varying in potential with structure of the substituent

Cyclic voltammetry scans for the ruthenium(II) complexes exhibit fundamentally a superimposition of that for the cationic ligands and 2,2'-bipyridyl associated ruthenium. The wave indicating the irreversible oxidation of the cationic ligand remains. This irreversible oxidation is presumably associated with an irreversible oxidative cleavage of the alkyl group from the quaternary nitrogen.

3.3 DNA Interaction

It has been established [6] that the 2,2'-bipyridyl bidentate ligand at ruthenium does not provide the capability for intercolation into double-stranded DNA. Such intercolation requires that the bidentate ligand associated with ruthenium bear additional functionality fostering intercolation, such as is provided through the use of the related bidentate ligand 1,10-phenanthroline. The ion $(phen)_3Ru^{2+}$ exhibits intercolative binding to calf thymus DNA by an intercolative action, whereas the structurally related $(bpy)_3Ru^{2+}$ does not. Particular binding of $(bpy)_2RuL_2^{2+}$ to calf thymus DNA would thus be seen as involving a different mode of interaction. The herein observed interaction with DNA of the synthesized species bearing cationic ligands L described above thereby is understood to occur through an alternative binding mechanism, particularly that involving interaction of the cationic sites on the ligands L with the anionic sites associated with the major groove of the double stranded DNA, of the type previously observed in our laboratories using polycationic "strings" of the type shown in Fig. 3 [1].

In a similar manner, emanating from the ruthenium center, cationic arms (L) are capable of interacting with the anionic major groove of the double stranded DNA and thereby holding the ruthenium system in closer contact with a stronger binding interaction with the DNA.

3.4 Fluorescence Spectra and DNA Binding

Verification of this concept for the new cationic ligands is obtained through the investigation of the fluorescence spectra of ruthenium complexes involving those ligands in the presence of double-stranded DNA. Binding constants for a series of the new ruthenium complexes have been measured from observation of the fluorescence spectra measured as noted in the Experimental (*vide infra*).

DNA binding characteristics of the newly prepared complexes were investigated using fluorescence titration analysis. The indicated newly prepared ruthenium complexes were investigated by titrating solutions of the specific ruthenium complex with another solution prepared to be equimolar in the ruthenium complex but with an excess of calf thymus DNA present. Excitation was performed by irradiation using 475 nm light and subsequent changes in the integrated fluorescence intensity (ΔF) were determined and used to calculate binding constants for the complexes bearing particular ligands using a modified Eadie-Hofstee method involving plotting ΔF *versus* $\Delta F/[BP]$ where [BP] is the DNA base-pair concentration.

Of the newly synthesized complexes investigated, most were found to be weak emitters when excited using wavelengths matching the visible region metal-to-ligand charge transfer (MLCT) absorptions. For many of these complexes, significant increases in such emissions were observed upon addition of calf thymus DNA at DNA base-pair to ruthenium complex ratios exceeding s factor of 20, thus indicating qualitatively that binding to double-stranded DNA was occurring. In certain instances, quantitative analysis of these data in attempts to determine binding constants using the method cited above were unsuccessful in providing consistent answers. This is presumably the result of the existence of several modes of

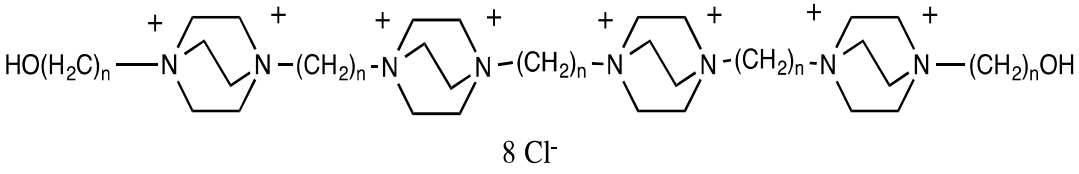


Fig. 3. General structure of "polycationic strings" - Cationic sites are located regularly along a flexible chain and thereby capable of interacting electrostatically with the (anionic) major groove of double stranded DNA

Binding for the complexes with DNA. An indication of this phenomenon was provided by the variability of the fluorescence peak positions and intensities as the DNA concentration was changed.

Results of a quantitative nature could be extracted for complexes (13), (17), and (19), (13) and (19) differing only in the nature of the bidentate ligand involved, (bpy) for the former and (phen) for the latter, with the same monodentate ligand (4). For (17) the bidentate ligand is (bpy) and the monodentate ligands are the *m*-methoxybenzyl system (8).

For the complexes of (13) and (17), both with the general formula $Ru(bpy)_2L_2^{4+}$, binding constants of 5 $x10^3$ and $4.2x10^3$ were measured respectively. As an example, the fluorescence spectra for the system from (13) are shown in Fig. 4, along with the mode of calculation of the binding constant. Previous investigations of $Ru(bpy)_3^{2+}$ and $Ru(phen)_3^{2+}$ provided binding constants of $1.4x10^3$ and $6.2x10^3$ respectively, and are consistent with our own measurements on the newly prepared complexes under the conditions reported above. [7] It should be noted that these binding constants are based upon base-pair concentrations for DNA. Therefore, the binding constants measured for complexes from (13) and (17) are indicative of an increased tendency toward binding for the bpy based complexes as reported here compared to $Ru(bpy)_3^{2+}$, a condition attributable to the additional pair of charged sites present on the monodentate 4,4'-bpy ligands.

For the complex from (19), with phen present in place of bpy, but bearing identical 4,4'-bpy based ligands, a binding constant of 8.2 $x10^3$ was determined. This additional increase in binding capability as compared to that for [13] is likely due to a greater tendency of phen to facilitate binding to the double stranded DNA.

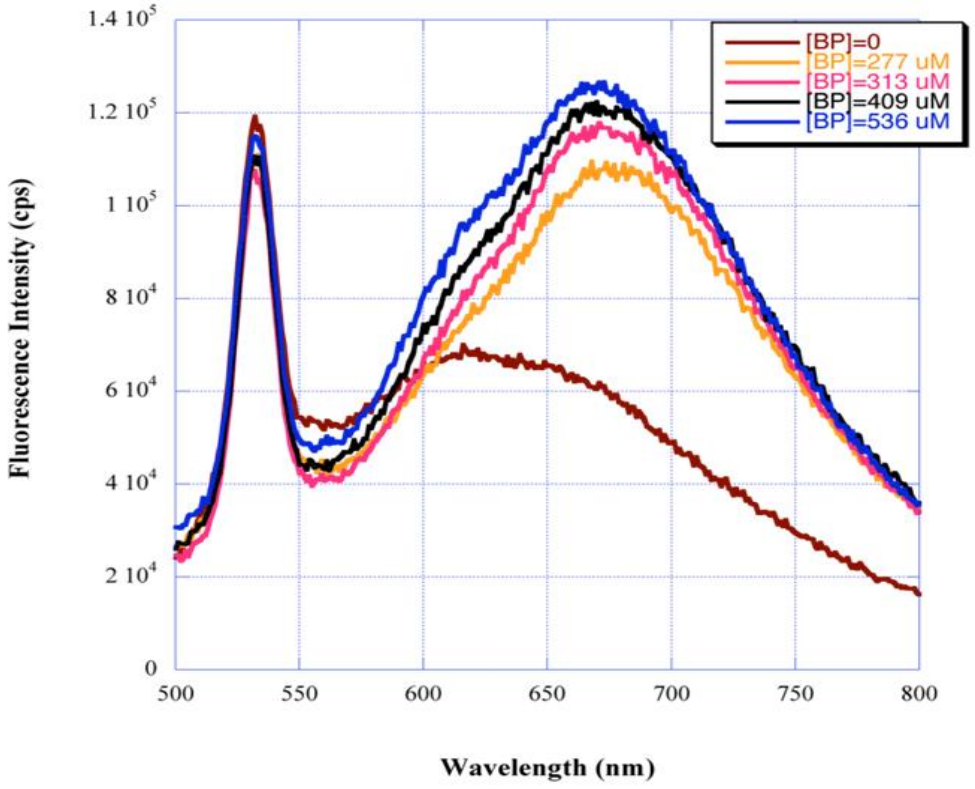


Fig. 4. Fluorescence spectra for complex from (13) using 475 nm excitation for [BP] = 0 and 277, 313, 409, 536 μM BP

Note: the sharp peak at 530 nm is the Raman line of water, indicating how weak the fluorescence signal is for [13]. The area from 500 to 800 nm under each spectrum was determined (F) and the increase for each BP concentration determined (ΔF). A plot of ΔF vs. ΔF/[BP] yielded a straight line with slope of $-(1/K_b)$,[8] thus determining $K_b = 5x10^3$

4. CONCLUSION

In light of these binding measurements, it would appear that the original concept that use of ligands bearing additional charged sites distant from the Ru center would enhance binding to DNA has been verified. Efforts are in progress for the design and synthesis of additional polycationic ligands for complexation with ruthenium (II) centered species with a view toward further increased ability for association with DNA. These results are also encouraging for further work to be involved with the design and syntheses of other species involving specifically cationic ligands where binding to DNA is involved with the biological activity of the species. An example of these systems is that of the square-planar platinum complexes, wherein the incorporation of cationic ligands might be anticipated to favor binding to DNA and thereby increased activity.

ACKNOWLEDGEMENT

The authors wish to recognize the support for this project from the PSC-BHE Research Award program, and for one of us (JH) support from the National Science Foundation in the form of a graduate fellowship,

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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