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# Synthesis, characterization and DNA binding affinities of water-soluble benzoheterocycle triosmium clusters

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**Abstract**

The syntheses of the water-soluble clusters $[Os_3(CO)_9(\mu\text{-}\eta^2\text{-}(L\text{–}H))(\mu\text{-}H)L']$ (L = 3-amino quinoline, $L' = Na_3[P(C_6H_4SO_3)_3]$ **1**; L = 3-amino quinoline, $L' = [P(OCH_2CH_2NMe_3)_3]I_3$, **2**; L = 3-(2-phenyl acetimido) quinoline, $L' = [P(OCH_2CH_2NMe_3)_3]I_3$, **3**; L = phenanthridine, $L' = [P(OCH_2CH_2NMe_3)_3]I_3$, **4**) are reported. The products have been fully characterized by $^1H$-, $^{13}C$ VT-NMR and LC–TOF-MS. The effect of pH and concentration on intermolecular aggregation in water has been investigated. The interactions of these clusters with DNA have been studied using the plasmid super coiled DNA relaxation test in a 1% electrophoresis agarose gel. Band retardation due to cluster binding was observed for the positively charged clusters **2**–**4**, but not for the negatively charged **1**. The relative binding affinities were in the order $\mathbf{2} < \mathbf{3} \sim \mathbf{4}$ and all three showed greater binding affinities than the positively charged cluster $[Rh_3(\mu_3\text{-}S)_2(\eta^5\text{-}Me_5C_5)_3](BF_4)_2$. These preliminary results suggest a relationship between the nature of the heterocyclic ligand and the binding affinity of the cluster to DNA.


*Keywords:* Metal clusters; DNA; Water soluble; Osmium

## 1. Introduction

The use of metal complexes to elucidate the structure and function of biological systems is an area of intense investigation [1–6]. Initial applications of such complexes involved their infusion into protein lattices as an aid in crystal structure determinations. Later covalent tethering of metal complexes to proteins has been used for this same purpose or for more general labeling purposes using solution phase spectroscopic techniques [1–3]. In a separate area of investigation, metal complexes are attached to organic molecules that are ligands for specific protein receptor sites [4]. More recently, certain complexes of heteroaromatics have proven to be somewhat selective for binding to DNA where subsequent photolysis-oxidative electron transfer induces cleavage of the bio-macromolecule [5,6]. In pursuing the development of all of the applications of metal complexes to the areas of biomedical interest outlined above, polymetallic complexes have a particularly active role to play because of the possibility of direct visualization using electron microscopy [7].

We have recently developed synthetic procedures for a novel class of electron deficient complexes of biomedically important benzoheterocycles with triosmium clusters of general formula $[Os_3(CO)_9(\mu_3\text{–}\eta^2\text{-}(L\text{–}H))(\mu\text{-}H)]$ (L = quinoline, 5,6-benzoquinoline, phenanthridine, quinoxaline, 2-methyl benzimidazole, benzoxazole, benzothiazole, and 2-methyl benzotriazole) [8–14]. These complexes can be synthesized in moderate to good yield by the reaction of $[Os_3(CO)_{10}(CH_3CN)_2]$ with the heterocycle at ambient temperature followed by photolytic decarbonylation of the intermediate decacarbonyl complex (for example quinoline see Scheme 1) [8].

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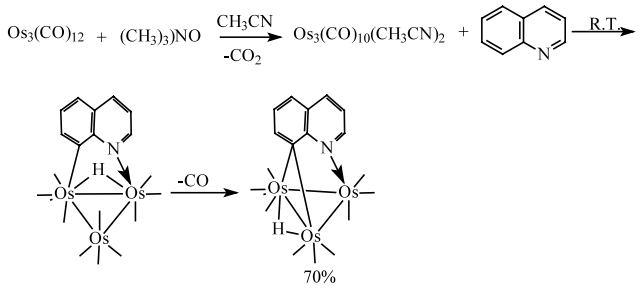



Scheme 1.

In addition to containing ligands that are already known binding agents to DNA, proteins, and neurotransmitter receptor sites [15–21], the novel methods developed for modifying these systems allow the synthesis of cluster complexes that are particularly well suited for development as markers for bio-macromolecules for the following reasons:

- The presence of the trimetallic core allows direct extraction of phase information from single crystal and low angle X-ray studies when the group interacting with the bio-macromolecule is bound to a trimetallic core [7].
- Positive and/or negative charges can be placed on the metal core and on the ring to impart water solubility. The resulting charged clusters should be stable in water over a wide range of pH owing to the air and thermal stability of the triosmium framework [8,11,14].
- The synthesis of complexes with like charges on both the ring and metal should prevent aggregation of these clusters in aqueous solution, allowing for more molecule specific interactions and sharper spectroscopic signals.
- Direct visualization with electron microscopy is possible.
- The target clusters possess carbonyl and hydride ligands that have infrared and NMR signals in regions where proteins and polynucleotides are silent.
- The synthetic methods developed for modifying the structure of the benzoheterocycle ring will allow extension of the ring systems to increase binding by intercalation and the construction of peptide tethers. Both of these types of modifications will enhance binding and specificity [9,10].

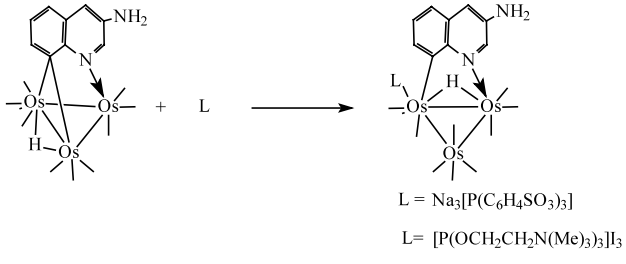
We report here our initial investigations into the development of these systems as bio-markers for DNA, which demonstrate some of the above potentialities, and where structure binding affinity relationships are beginning to emerge.

## 2. Results

### 2.1. Synthesis and characterization of water-soluble quinolyl triosmium clusters

We began our studies with the synthesis of water-soluble phosphine derivatives of the previously reported $[Os_3(CO)_9(\mu_3-\eta^2\text{-}(L-H))(\mu\text{-}H)]$ (L = 3-amino quinoline) [11]. This complex was chosen because it contains a good hydrogen bond donor–acceptor that could enhance binding to biomacromolecules and because the amino group is amenable to further modification. In order to understand the impact of charge on the aqueous behavior of a given water-soluble cluster we reacted the electron deficient quinolyl cluster with the commercially available, negatively charged water solubilizing phosphine $Na_3[P(C_6H_4SO_3)_3]$ and with the positively charged $[P(OCH_2CH_2NMe_3)_3]I_3$ which was synthesized by reaction of $[P(OCH_2CH_2NMe_2)_3]$ with methyl iodide. The latter was chosen because its positive charge is equal in magnitude to the negative charge on $Na_3[P(C_6H_4SO_3)_3]$ and because its charge should be independent of pH. Both ligands react quantitatively with the deep green quinolyl complex when an aqueous solution of the phosphine is added to a methanol or acetone solution of the cluster yielding $[Os_3(CO)_9(\mu-\eta^2\text{-}(L-H))(\mu\text{-}H)L]$ (L = 3-amino quinoline, L′ = $Na_3[P(C_6H_4SO_3)_3]$, **1**; L = 3-amino quinoline, L′ = $[P(OCH_2CH_2NMe_3)_3]I_3$, **2**, Scheme 2). The resulting yellow orange adducts were characterized by LC–MS, $^1$H- and $^{13}$C-NMR and elemental analysis. The ESI–MS shows singly, doubly and triply charged ions in the negative ion spectrum for **1** at 1464, 730 and 470 amu all showing the isotopic distribution patterns associated with the triosmium cluster fragments. The peaks centered at 1464 corresponds $[M^{3-}+2Na^{+}-CO]^{-}$, the peak at 730 corresponds to $[M^{3-}+Na^{+}-CO]^{2-}$ and the peak at 470 corresponds approximately to $[M^{3-}-CO]^{3-}$. The corresponding positive ion cluster fragments for **2** are observed at 1565, $[M^{3+}+2I^{-}]^{+}$, 717, $[M^{3+}+I^{-}]^{2+}$ and 417 amu $[M^{3+}-2CO]^{3+}$.

The $^1$H-NMR of **1** and **2** both show doublet hydride resonances at −12.03 ppm ($J_{PH}$ = 16 Hz) and −12.97 ppm ($J_{PH}$ = 16 Hz). The magnitude of these coupling constants suggests that the phosphine is located on one




Scheme 2.

of the two osmium atoms bridged by the hydride. The $^{13}$C-NMR of an aqueous solution of a $^{13}$CO-enriched sample of **2** in the carbonyl region reveals a pattern of carbonyl resonances that is very similar to that observed for the corresponding triphenylphosphine-quinoline adduct whose solid state structure has been determined [8,22]. Both **2** and the triphenylphosphine adduct show two phosphorous coupled resonances of relative intensity one at 185.6 ($J_{PC} = 7.9$ Hz) and 184.3 ppm ($J_{PC} = 5.0$ Hz) for **2** and at 185.64 ($J_{PC} = 6.2$ Hz) and 182.5 ppm ($J_{PC} = 4.6$ Hz), respectively. Both also show a set of partially overlapping singlet resonances of total relative intensity six in the region 177.4–181.3 ppm. Based on this data we propose structures for both **1** and **2** where the phosphine is located on carbon bound osmium *cisoid* to the hydride ligand (Scheme 2).

The $^{1}$H- and $^{13}$C-NMR resonances for **1** and **2** are significantly broadened in aqueous solution suggesting that these compounds form micelles or related aggregates in solution. This is particularly true for the aromatic resonances of the heterocyclic ligand. In the case of **1** a slow isomerization process is observed (over the course of 24 h) as evidenced by the fact that the initially observed doublet hydride resonance at −12.03 ppm gradually decreases in intensity and new sharp doublet resonances at −12.25 and −12.65 ppm and a very broad singlet resonance appear at −15.6 ppm. This can be attributed to a dissociative isomerization (probably within the aggregate) to yield the possible regioisomers of the phosphine adduct (Scheme 3). An analogous series of isomers has been previously observed in related triosmium clusters [22]. When aqueous solutions of relatively high concentrations of **1** are allowed to stand at room temperature the dark green, $[Os_3(CO)_9(\mu_3\text{–}\eta^2\text{-}(L\text{–}H))(\mu\text{-}H)]$ (L = 3-amino quinoline) gradually precipitates. This observation seems to corroborate the proposed dissociative isomerization and seems to indicate that the bulky triphenylphosphine sulfonate would rather aggregate with itself than as the complex **1**.

In sharp contrast, **2** shows no tendency to isomerize in aqueous solution. Lowering the pH from 7 to 5 leads to significant sharpening of the aromatic heterocycle ligand resonances (Fig. 1). The p$K_a$ of the anilinic nitrogen is ca. 4.6; therefore changing the pH from 7 to 5 lowers the free amine to protonated amine ratio from 200 to 1 to 2 to 1. This increase in the degree of protonation is sufficient to decrease the level of aggregation by placing like charges on the heterocyclic ligand and the ancillary phosphine ligand. Interestingly, although the aromatic are significantly broadened in **1** and **2**, the hydride resonances are relatively sharp. This is probably due to the relatively long relaxation times of the hydrides in these complexes compared with the aromatic protons [12].

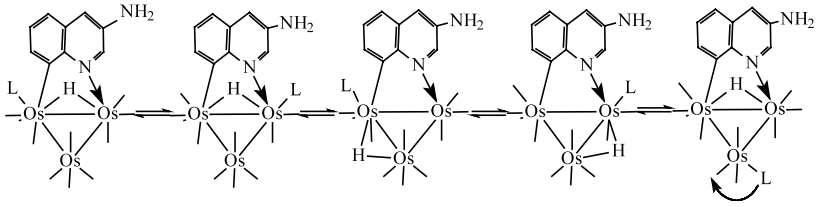
Prior work has shown that extending aromatic ring system from two to three rings greatly improves its ability to intercalate into the major groove of DNA [5,6]. Therefore, we chose to extend the aromatic ring system in **2** by tethering a 2-phenyl acetyl group to the 3-amino quinoline and by utilizing the tricyclic ring system phenanthridine (Scheme 4). Both of these systems reacted quantitatively with $P(OCH_2CH_2NMe_3I)_3$ to provide the desired water-soluble clusters $[Os_3(CO)_9(\mu\text{–}\eta^2\text{-}(L\text{–}H))(\mu\text{-}H)L]$ (L = 3-(2-phenylacetamido) quinoline, L′ = $[P(OCH_2CH_2NMe_3)_3]I_3$, **3**; L = phenanthridine, L′ = $[P(OCH_2CH_2NMe_3)_3]I_3$, **4**, (Scheme 4). Their $^{1}$H-NMR indicated that they are isostructural with regard to the location of the phosphine relative to **2**.

### 2.2. Plasmid DNA binding affinity studies

The plasmid relaxation test was conducted using commercially available pUC19 plasmid DNA. Upon incubation with the cluster, the super-coiled form can unwind to the open circular shape or, if double strand cleavage is promoted, it can open to linear double stranded DNA. Any modification in shape and/or charge can be accurately detected in a 1% agarose gel and by subsequent gel staining with ethidium bromide.

A concentration gradient of three different water-soluble clusters was incubated with the plasmid in order to understand whether any interaction occurred. The incubation time was 1 h at 37 °C and the different concentrations were run against two controls, one being the untreated plasmid and the other being the linear DNA obtained by digestion of the plasmid with the restriction enzyme BamH*I*.

As expected, the negatively charged cluster **1** showed no evidence of binding to the plasmid due to the repulsive interaction with the negatively charged DNA backbone. In order to examine the strictly electrostatic component of cluster binding, the model compound



Scheme 3.

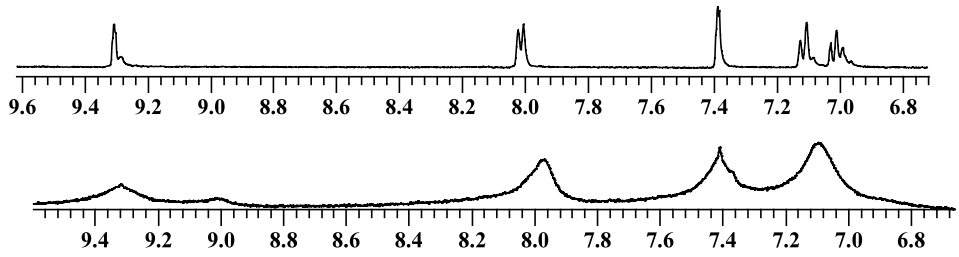


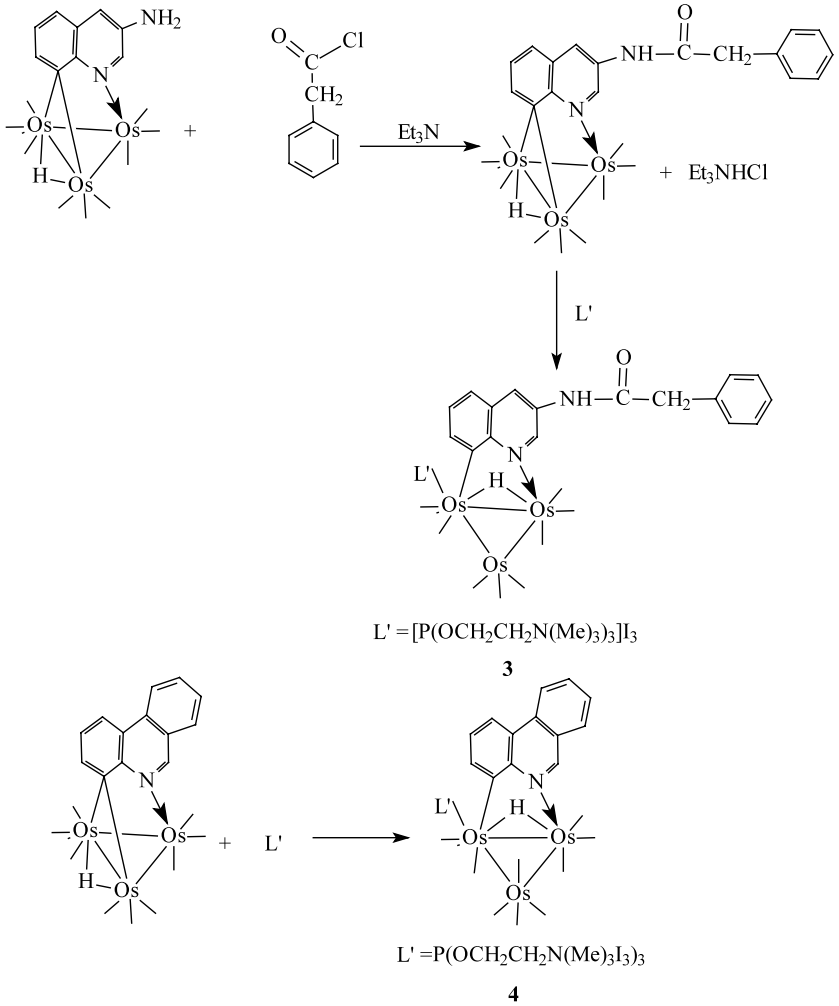

Fig. 1. $^1$H-NMR of the aromatic resonances of **2** at 400 MHz in $D_2O$ at pH 5 (top), $^1$H-NMR of the aromatic resonances of **2** at 400 MHz in $D_2O$ at pH 7 (bottom).

$[Rh_3(\mu_3\text{-}S)_2(\eta^5\text{-}Me_5C_5)_3]^{2+}$ was tested first as its tetrafluoroborate salt [23]. Band retardation was observable at 375 μM and increased until 1 mM when precipitation of the DNA cluster complex in the well occurred (Fig. 2)

The complex **2** was then tested in an identical manner. Interaction is again observed in the form of band retardation with respect to the unaltered DNA. This means that the DNA mass/charge ratio has changed upon incubation with the cluster. Significant band retardation was observable at 41 μM and precipitation occurred at 50 μM (Fig. 3). This represents an approximately ninefold increase in binding affinity relative to $[Rh_3(\mu_3\text{-}S)_2(\eta^5\text{-}Me_5C_5)_3]^{2+}$.

The 'dangling phenyl ring', water-soluble cluster, **3**, exhibited an even stronger binding affinity than **2**. Band retardation was observable at 8.2 μM and precipitation was complete at 33 μM representing a approximately fivefold increase in binding affinity relative to **2** (Fig. 4).

Finally, we tested the binding affinity of the phenanthridine complex **4**, which has a more extensive ring system than **2** but a more rigid fused three-ring system relative to **3** and therefore may interact with DNA in a




Scheme 4.

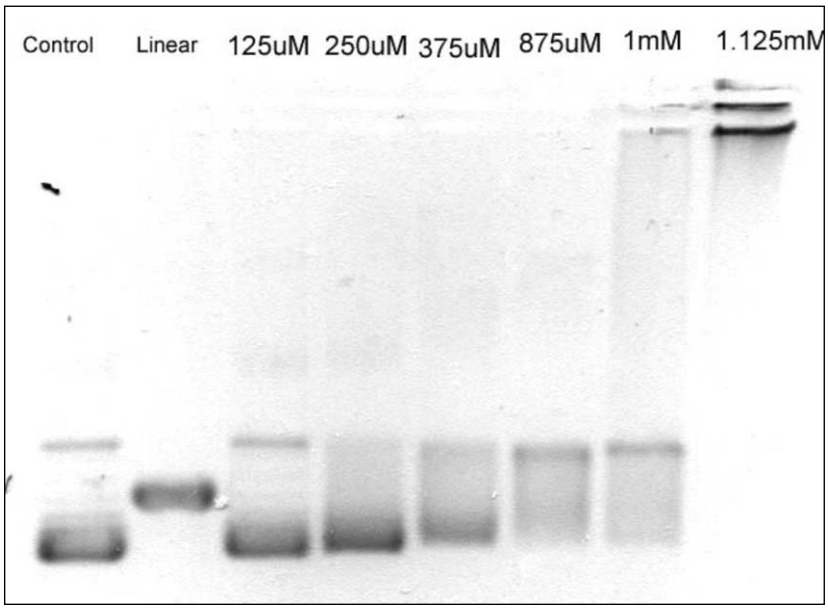



Fig. 2. Ethidium bromide developed plasmid relaxation test agarose gel incubated with various concentrations of $[Rh_3(\mu_3\text{-}S)_2(\eta^5\text{-}Cp^*)_3](BF_4)_2$.

different fashion. The degree of band retardation observed at 41.2 for **2** and at 8.2 μM for **3** in the previous experiments is now present at the concentration of 24.7 μM (Fig. 5). Precipitation was apparently complete at 33 μM as for **3**.

## 3. Discussion

The plasmid relaxation test was chosen for the bioassay of **1**–**4** for multiple reasons:

- The high sensitivity of the test allows working at very low concentrations of DNA and cluster.
- Facile DNA detection can still be obtained at those concentrations by staining the gel with ethidium bromide after electrophoresis, so avoiding possible interference when running the gel.
- Multiple cluster effects on DNA can be detected in the gel.

The main result we wanted to obtain from these tests was the actual detection of any form of interaction

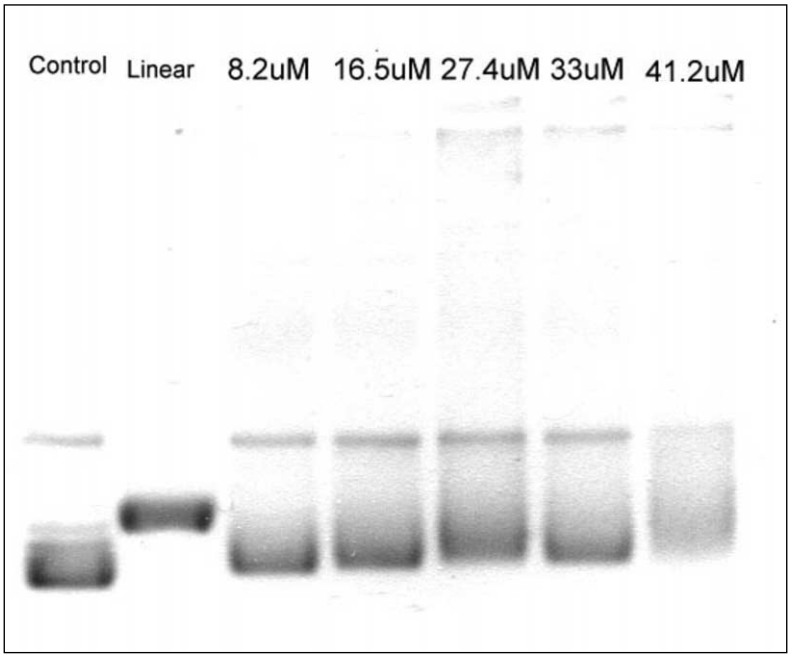



Fig. 3. Ethidium bromide developed plasmid relaxation test gel of $Os_3(CO)_9(\mu\text{–}\eta^2\text{-}(3\text{-}NH_2)C_9H_5N)(\mu\text{-}H)([P(OCH_2CH_2N(Me)_3)_3]I_3)$ (**2**).

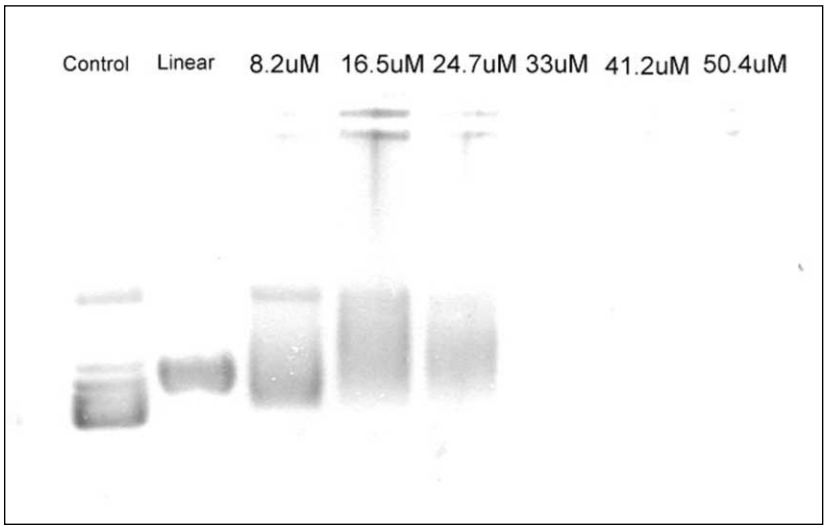



Fig. 4. Ethidium bromide developed plasmid relaxation test gel of $Os_3(CO)_9(\mu-\eta^2$-(3-NH $COCH_2C_6H_5)C_9H_5N)(\mu$-H)([$P(OCH_2CH_2N(Me)_3)_3]I_3$) (**3**).

between our clusters and a reproducible and constant form of DNA, such as a plasmid. In this regard, other double stranded forms of DNA, such as calf thymus, are not suitable because the extreme heterogeneity prevents any precise and accurate analysis.

Our results do not allow an unequivocal characterization of the interaction observed, but an important point can be made: whatever interaction is occurring upon incubation, the cluster concentration at which it can be observed is different for different clusters. As the charge on the water-soluble ligand is constant in all the experiments with **2**–**3**, the heterocycle must be involved in the observed effect because the heterocycle is the only part of the cluster that changes in the experiment.

As the most reactive cluster features a dangling 'arm' comprised of a phenyl ring connected to the heterocycle by a peptide linkage, we believe that the presence of a flexible ligand remote enough from the bulky triosmium frame is a critical factor in enhancing the ability of the cluster to adapt and bind to the DNA structure.

In order to achieve such flexibility, it is essential to increase the number of individually solvated molecules: our solubility studies have shown that like charges in different portions of the molecule succeed in breaking

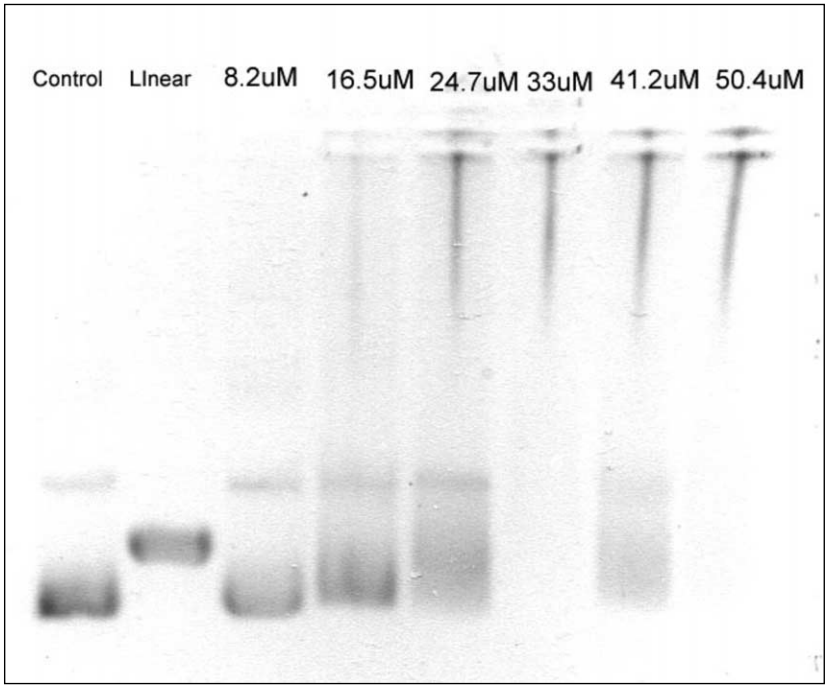



Fig. 5. Ethidium bromide developed plasmid relaxation test gel of $Os_3(CO)_9$ ($\mu-\eta^2$-$C_{13}H_8$([$P(OCH_2CH_2N(Me)_3)_3]I_3$) (**4**).

up the aggregation processes happening at neutral pH in aqueous solutions.

In light of these results, heterocycles with a positive charge at neutral pH and containing a flexible aromatic ring tether represent a synthetic goal we will pursue in our research.

## 4. Experimental

The complexes, $[Os_3(CO)_9(\mu-\eta^2\text{-}(L-H))(\mu\text{-}H)]$ (L = 3-amino quinoline, phenanthridine) were synthesized according to literature procedures [9,10]. NMR spectra were obtained on a Varian Unity Plus 400 MHz spectrometer and IR spectra were obtained on a Thermo Nicolet 633 FT-IR. Elemental analyses were performed by Schwarzkopf Analytical Labs, Woodside, New York. LC–Electrospray–MS were run on a Micromass Time of Flight spectrometer. The cluster $[Rh_3(\mu_3\text{-}S)_2(\eta^5\text{-}Me_5C_5)_3](BF_4)_2$ was synthesized according to literature procedures [23].

### 4.1. Plasmid relaxation test

Agarose gels (1%) were prepared by dissolving agarose in double distilled water and by warming the solution at ~60 °C. The plasmid pUC19 was incubated with the appropriate amount of water-soluble cluster for 1 h at ~37 °C. After loading the wells, the gel was run for ~5 h at a potential of 37 V. The gel was subsequently soaked in a 1% solution (v/v) of ethidium bromide in double distilled water and destained in pure water overnight to obtain better light contrast and band definition. The gels were then photographed by shining UV light on the developed gel.

### 4.2. Synthesis of tris-(2-trimethylamino iodide)ethyl phosphite

In a 50 ml round-bottom flask, 5.22 ml of $[P(O(CH_2)_2N(Me)_2)_3]$ (United Organometallics) were dissolved in 20 ml of 95% EtOH at ~0 °C in an ice bath. Through a dropping funnel, 4.12 ml of MeI were added drop wise to the solution in the flask. A white precipitate formed and then 60 ml of $Et_2O$ were added to completely precipitate the alkylated product from the solution. The precipitate was vacuum filtered and dried overnight under vacuum. Yield: 11.2 g (89.6%). (MW = 739 g mol$^{-1}$). $^1$H-NMR ($D_2O$), $\delta$: 4.26 (m, 2H), 3.56 (t, 2H), 3.08 (s, 9H). Anal. Calc. for $C_{15}H_{39}I_3N_3O_3P$: C, 25.3; H, 5.8; N, 5.5. Found: C, 25.08, H, 5.87, N, 5.7%.

### 4.3. Synthesis of $[Os_3(CO)_9(\mu-\eta^2\text{-}(3\text{-}NH_2)C_9H_5N)(\mu\text{-}H)(Na_3[P(C_6H_4SO_3)_3])]$ (1)

In a 25 ml round bottom flask, were placed 50 mg of $[Os_3(CO)_9(\mu-\eta^2\text{-}(3\text{-}NH_2)C_9H_5N)(\mu\text{-}H)]$ (MW = 966.87 g mol$^{-1}$) and 10 ml of MeOH (or $C_3H_6O$). An equimolar amount (30 mg of $Na_3[P(C_6H_4SO_3)_3]$ (Aldrich, MW = 568.4 g mol$^{-1}$), in a few drops of water is then added to the cluster solution. The reaction is quantitative and yields 80 mg (100%) of the yellow product, $[Os_3(CO)_9(\mu-\eta^2\text{-}(3\text{-}NH_2)C_9H_5N)(\mu\text{-}H)(Na_3[P(C_6H_4SO_3)_3])]$ (**1**) (MW = 1535.27). IR ($\nu$CO, $D_2O$): 2006 (s), 2139 (m), 2169 (w), 2194 (w), 2236 (m), 2273 (sh), 2297 (s), 2318 (s), 2339 (s) cm$^{-1}$. $^1$H-NMR data ($D_2O$), $\delta$: 9.28 (s, 1H), 8.13 (d, 1H), 7.43 (s, 1H), 7.36 (m, 12H), 7.08(d, 1H), 7.02 (t, 1H), −13.01 (d, 1H, $J_{PH}$ = 16.0 Hz). Anal. Calc. for $C_{36}H_{20}N_2Na_3Os_3P$: C, 28.16; H, 1.3; N, 1.8. Found: C, 28.53; H, 1.94; N, 1.82%.

### 4.4. Synthesis of $[Os_3(CO)_9(\mu-\eta^2\text{-}(3\text{-}NH_2)C_9H_5N)(\mu\text{-}H)([P(OCH_2CH_2N(Me)_3)_3]I_3)]$ (2)

In a 25 ml round bottom flask 50 mg of $[Os_3(CO)_9(\mu_3-\eta^2\text{-}(3\text{-}NH_2)C_9H_5N)(\mu\text{-}H)]$ (0.05 mmol) were dissolved in 10 ml of MeOH (or $C_3H_6O$), then an equimolar amount of $[P(O(CH_2)_2N(Me)_3)_3]I_3$, 38.2 mg, dissolved in a few drops of water was added to the cluster solution. The reaction is quantitative and yields ~88 mg (100%) of yellow product (MW = 1705.87). IR ($\nu$CO, $D_2O$): 2013 (s), 2139 (m), 2172 (w), 2205 (w), 2228 (sh), 2275 (sh), 2317 (s), 2342 (s), 2358 (s) cm$^{-1}$. $^1$H-NMR data ($D_2O$), $\delta$: 9.30 (s, 1H), 8.1 (d, 1H), 7.4 (s, 1H), 7.12 (d, 1H), 7.02 (t, 1H), 2.9 (s, 27H), 3.4 (m, 6H), 3.07 (s, 3H), 3.04 (s, 3H), −12.97 (d, 1H, $J_{PH}$ = 16 Hz)). $^{13}$C-NMR ($D_2O$), $\delta$: 177.1 (s), 178.2 (s), 179.2 (s), 180.2 (s), 181.1 (s), 184.2 (d, $J_{PC}$ = 5.0 Hz), 185.1 (d, $J_{PC}$ = 7.9 Hz), 186.1 (s). Anal. Calc. for $C_{33}H_{47}I_3N_5O_{12}Os_3P$: C, 23.0; H, 2.35; N, 4.1. Found: C, 22.84; H, 2.13; N, 3.97%.

### 4.5. Synthesis of $[Os_3(CO)_9(\mu_3-\eta^2\text{-}(3\text{-}NH\text{-}CO\text{-}CH_2\text{-}C_6H_5)C_9H_5N)(\mu\text{-}H)]$

$Os_3(CO)_9(\mu_3-\eta^2\text{-}(3\text{-}NH_2)C_9H_5N)(\mu\text{-}H)$, 50 mg (0.05 mmol) were dissolved in 10 ml of tetrahydrofuran. Triethyl amine, 7 ml and then 6.6 ml of phenyl acetyl chloride, were added, both in stoichiometric amounts. The reaction was deemed to be complete when triethyl ammonium chloride, the reaction byproduct, precipitates in the solution. After filtration, the solvent is rotary evaporated and the product recovered as a green solid. The reaction is made quantitative by adding a 10% excess of trimethylamine, which can be easily removed during the solvent evaporation step. Yield: 60 mg (100%) IR ($\nu$CO, $C_6H_{14}$): 2072 (m), 2040 (m), 2011 (s),

2001 (s), 1978 (sh), 1962 (s), 1953 (s), 1926 (s) $cm^{-1}$. $^1$H-NMR, ($D_2O$): $\delta$: 9.7 (s, 1H), 8.7(d, 1H), 8.6 (s, 1H), 8.4 (dd, 1H), 7.34 (m, 5H), 7.2 (t, 1H), 4.2 (s, 2H) −11.97 (s, 1H). Anal. Calc. for $C_{26}H_{14}N_2O_{10}Os_3$: C, 28.78; H, 1.29; N, 2.58. Found: C, 29.01; H, 1.40; N, 2.61%.

### 4.6. Synthesis of $[Os_3(CO)_9(\mu$-(3-NH–CO–$CH_2$–$C_6H_5)C_9H_5N)(\mu$-H) ($[P(OCH_2CH_2N(Me)_3)_3]I_3$)] (**3**)

Fifty milligrams of $Os_3(CO)_9(\mu_3-\eta^2$-(3-NH–CO–$CH_2$–$C_6H_5)C_9H_5N)(\mu$-H) were dissolved in ~10 ml of MeOH (or $C_3H_6O$) and then a stoichiometric amount of $[P(OCH_2CH_2N(Me)_3]I_3$ was dissolved in a few drops of water and then added to the cluster solution. The reaction is quantitative. Yield: 62 mg (100%) IR ($\nu$CO, $D_2O$): 2086 (w), 2073 (w), 2042 (sh), 2020 (sh), 1996 (s), 1974 (s), 1959 (s), 1938 (s) $cm^{-1}$. $^1$H-NMR, ($D_2O$), $\delta$: 9.31 (s, 1H), 8.12 (d, 1H), 7.42 (s, 1H), 7.34 (m, 5H), 7.12 (d, 1H), 7.02 (t, 1H), 4.1(s, 2H), 3.41 (m, 6H), 3.07 (s, 3H), 3.04 (s, 3H), 2.91 (s, 27H), −12.97 (d, 1H, $J_{PH}$ = 15.89 Hz). Anal. Calc. for $C_{41}H_{53}I_3N_5O_{13}Os_3P$: C, 28.97; H, 3.23; N, 3.83. Found: C, 28.52; H 3.33; N, 3.85%.

### 4.7. Synthesis of $[Os_3(CO)_9(\mu-\eta^2$-$C_{13}H_8N)(\mu$-H) ($[P(OCH_2CH_2N(Me)_3]I_3)$] (**4**)

The same procedure as for **2** was used. Yield: 63 mg (100%). IR ($\nu$CO, $D_2O$): 2075 (w), 2073 (w), 2038 (sh), 2025 (sh), 1991 (s), 1967 (s), 1961 (s), 1945 (s) $cm^{-1}$. $^1$H-NMR, ($D_2O$), $\delta$: 9.45 (d, 1H), 8.9 (d, 1H), 8.31 (d, 1H), 7.92 (s, 1H), 7.41 (dd, 1 H), 7.36 (dd, 1H), 7.16 (dd, 1H), 7.10 (d, 1H), 3.42 (m, 6H), 3.07 ppm (s, 3H), 3.04 (s, 3H), 2.91 (s, 27H), −11.89 (d, 1H) ($J$ $^{31}P-^1H = 15.87$ Hz). Anal. Calc. for: $C_{37}H_{48}I_3N_4O_{12}Os_3P$: C, 27.22; H 3.05; N, 3.17. Found: C, 27.0; H, 3.03; N 3.21%.

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