Journal of Organometallic Chemistry 734 (2013) 53–60

Contents lists available at SciVerse ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Relative rates of reaction of Pt(en)Cl $(NH_2R)^+$ with guanosine monophosphate as a function of amino group substituent: Toward efficient labeling of DNA for TEM imaging

Rakesh Kumar [a], Edward Rosenberg [a,*], Miriam Inbar Feske [b], Antonio G. DiPasquale [c]

[a] Department of Chemistry, University of Montana, Missoula, MT 59812, USA
[b] Latham and Watkins, Menlo Park, CA 94025, USA
[c] Department of Chemistry, University of California, Berkeley, CA 94720, USA

ARTICLE INFO

Article history:
Received 28 September 2012
Received in revised form
30 October 2012
Accepted 1 November 2012

Keywords:
Platinum complexes
DNA
TEM
dGMP
Osmium clusters

ABSTRACT

In an attempt to understand the factors that govern the rates of reaction of the complexes [Pt(en)Cl$(NH_2R)]^+NO_3^-$ (en = ethylene diamine) with guanosine monophosphate (dGMP) a series of amine complexes, where R=$C_8H_9NO_2$ (benzo[*d*][1,3]dioxol-5-ylmethanamine) (**1**), $C_8H_{11}N$ (phenethylamine) (**2**), C_7H_9N (benzylamine) (**3**), C_6H_7N (aniline) (**4**), C_6H_6IN (*p*-iodo-aniline) (**5**) C_3H_9NO (2-methoxy-ethylamine) (**6**) and $C_6H_{13}N$ (cyclohexylamine) (**7**), were synthesized and their reactions with deoxy-guanosine monophosphate (dGMP) were followed by ^1H NMR. Compound **1** was initially chosen because it showed significant water solubility. Compound **1** reacted quantitatively but slowly with dGMP and a subsequent Transmission Electron Microscopy (TEM) study of the binding **1** to a GATC DNA repeat gave a TEM micrograph that showed selective labeling of DNA at guanine, using a technique that allowed the laying down of a straight single strand of DNA on a carbon platform. The TEM suggested a possible side reaction with adenine and so a study of the reaction of **1** with adenine was performed and showed slow and what appeared to be non-specific binding to deoxyadenosine monophosphate (dAMP). The reactions of compounds **2**–**7** with dGMP were then studied by ^1H NMR and it was found that **2** reacted much faster than **1** with dGMP while the remaining complexes reacted more slowly. No reaction of **2** with dAMP was observed in the same time frame. The ultimate goal of the project was to bind a third row transition metal cluster to guanine and given the effective binding of **1** to DNA the synthesis of the complex $[Os_3(CO)_{11}PPh_2(CH_2)_2NH_2(en)PtCl]NO_3$ (**9**) is also reported that contains Pt as a linker to label guanine. The synthesis was performed by reacting $Os_3(CO)_{10}(CH_3CN)_2$ with $Ph_2PCH_2CH_2NH_2$ which gave an η^2 chelate complex $Os_3(CO)_{10}PPh_2(CH_2)_2NH_2$ (**8**). Complex **8** was reacted with [Pt(en)Cl(DMF)]NO_3 in a CO atmosphere to give **9**. ^1H and ^{195}Pt NMR indicate formation of an adduct with dGMP but too slowly to be of use in labeling DNA. The solid-state structure of **8** is also reported.

1. Introduction

Beer and Moudrianakis (1962) first suggested that it may be possible to sequence DNA with the aid of the electron microscope [1]. Transmission Electron Microscopy (TEM) has the potential to bring the base pair reading length up to 10^5 base-pairs per minute if the bases could be specifically labeled with heavy atoms. Long reading length enables detection of long repeating patterns in the genetic code and facilitates high-speed sequencing. Hence, the cost of DNA sequencing can be reduced significantly with the use of TEM. DNA has only light element (C,H,N,O,P), that are inherently transparent to TEM. Heavy metal ($Z > 70$) staining is well known to increase image contrast in TEM, and heavy metal salts have long been used in TEM to render nucleic acids visible [1–4]. The relative number of electrons that are scattered to a detector is approximately proportional to $Z^{1.5}$. The inherent drawback in using metal salts' staining is the lack of specificity to a nucleotide base owing to non-specific interactions. Therefore, covalent adduct formation between a DNA base and a metal complex is necessary for determination of the position of a specific base in a DNA molecule, Thus, functionalized heavy metal complexes capable of base-specific labeling of DNA can provide a tool for DNA sequencing. Furthermore, the labels need to have high reactivity to attach themselves specifically to one of the four bases, GATC, along with very high

* Corresponding author.
E-mail address: edward.rosenberg@mso.umt.edu (E. Rosenberg).

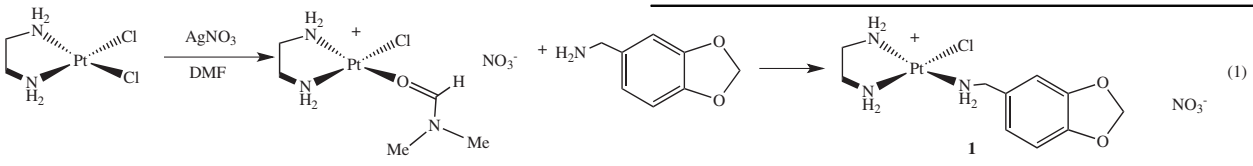
0022-328X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jorganchem.2012.11.003

specificity. The key to getting sequence information from the heavy atom labeling is the ability to stretch a single strand of DNA onto a platform where multiple images of the strands can be added together to give the exact sites of heavy atom labeling. Recently, such a technique has been developed, by Halcyon Molecular and provided the opportunity to develop heavy atom labels for DNA [5]. We have been studying the interaction of benzoheterocycle complexes of triosmium carbonyl clusters for the last ten years [6–8]. These studies revealed some of the structural requirements for selective binding of modified triosmium clusters with guanosine monophosphate (dGMP). Selectivity for guanine over the other bases was also observed [7,8]. However, subsequent attempts to obtain a straight and stretched single stranded DNA containing a covalently bound triosmium cluster failed. In order to demonstrate proof of concept for the technique of visualizing a guanine bound metal complex on DNA with TEM we studied the reaction of the complexes $[Pt(en)Cl(NH_2R)]^+NO_3^-$ with dGMP and DNA. It was previously been shown that complexes of this type were selective for binding to guanine when R was a luminescent molecule [9]. We report here the successful visualization of the Pt atoms in the complex $[Pt(en)Cl(NH_2R)]^+NO_3^-$ (R═$C_8H_9NO_2$ (benzo[*d*][1,3]dioxol-5-ylmethanamine, also known as piperonylamine) (**1**)) bound to DNA by TEM as well as a qualitative kinetic study of a series of related complexes reacting with dGMP and the synthesis and dGMP binding of a Pt-triosmium conjugate.

2. Results

2.1. Synthesis of *1* and its reaction with dGMP

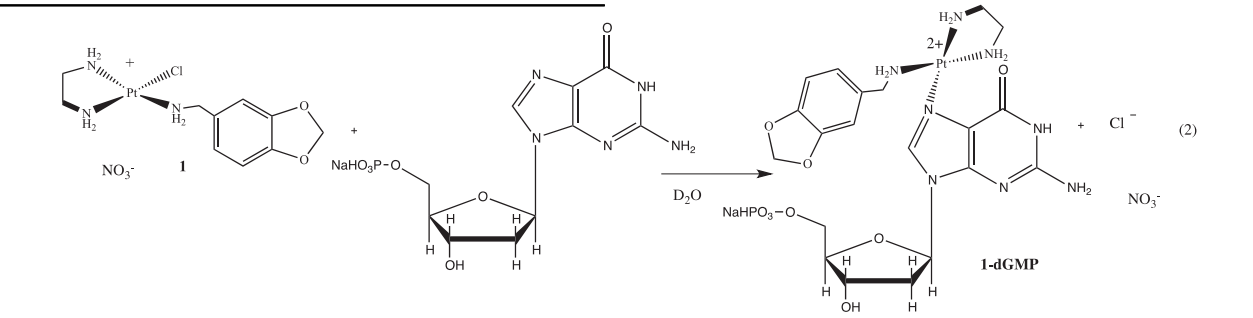
Complex **1** was synthesized by the reaction of benzo[*d*][1,3]dioxol-5-ylmethanamine with $[(en)PtCl_2]$ via halide abstraction in dimethylformamide (Equation (1)).



The complex was characterized by ^1H, ^{195}Pt NMR and mass spectrometry. The ^{195}Pt NMR of the DMF intermediate complex showed two overlapping resonances in DMF/THF-d^8 which we attribute to a mixture of the DMF and THF-d^8 complexes (Fig. 1a). To this solution was added 0.8 equivalents of piperonylamine, the solution evaporated to dryness and dissolved in D_2O. The ^{195}Pt NMR showed a single peak at −2625 ppm relative to chloroplatinic acid (Fig. 1b) [10]. To this solution of **1** was added an equimolar (based on the piperonylamine) amount of dGMP and a shift to −2511 ppm was observed (Fig. 1c). This gives evidence for the formation of a single adduct of **1** with dGMP.

2.2. The reaction of *1* with dGMP followed by ^1H NMR

Equimolar solutions of **1** and dGMP were combined in D_2O and the reaction followed by ^1H NMR over the course of 24 h using solvent suppression for the HDO peak (Fig. 2). Resonances assignable to residual DMF were observed at 8.3 ppm (CH) and 2.3 and 2.4 ppm (2 Me). Thirty minutes after addition of dGMP a new resonance appeared at 8.5 ppm downfield of the H(8) of dGMP at 7.9 ppm which we assign to the formation of the adduct **1-dGMP** (Equation (2)).



With increasing time the resonance at 7.9 ppm continues to decrease while the resonance at 8.5 ppm. After 19 h the resonance at 7.9 ppm has completely disappeared and only the peak assignable to **1-dGMP** is observed accompanied by the DMF resonance at 8.3 ppm in the downfield region of the spectrum. Other changes in the resonances above 7.0 ppm also observed.

2.3. Transmission electron microscopy of *1* incubated with ss-DNA GATC repeat

Based on these results **1** was incubated with a 25 mmol solution of a single-stranded GATC repeat (60 bases in length) at 40 °C for 6 h in aqueous phosphate buffer using a six-fold molar excess of label (compound **1**) relative to the number of guanines. After dialysis to remove excess label a micro drop of the solution was subjected to the stretching and straightening technique developed by Halcyon Molecular [5]. Multiple strands were deposited on glass slide, sprayed with carbon and imaged on the 300 kV VG Microscope at Oak Ridge National Lab [11]. Fig. 3 shows a TEM image of a segment of the labeled GATC repeat. There are 15 G bases in the segment using the calculated value 0.77 nm/base and 11 of them are labeled (green dots). There are several labels that are out of sequence (red dots) and these could be labeled adenines.