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N-Heterocyclic Carbenes Derived from Guanosine: Synthesis and Evidences of Their Antiproliferative Activity

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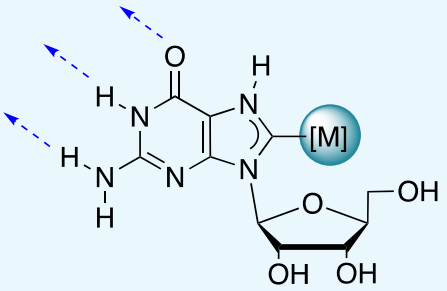
Supporting Information

ABSTRACT: Palladium(II) and platinum(II) complexes bearing N-heterocyclic carbenes derived from guanosine are synthesized via oxidative addition, followed by protonation in the presence of acid. Cytotoxicity of the compounds is evaluated in several cell lines. Compounds **2a**, **2b**, and **3a** are selective for glioblastoma U251 cells and are nontoxic toward healthy human embryonic kidney (HEK293) cells.



[M]= $Pd(PPh_3)_2Br$, $Pt(PPh_3)_2Br$

INTRODUCTION

N-heterocyclic carbenes[1] (NHCs) are widely used as ligands for transition metals, with extensive applications in catalysis[2] and medicinal chemistry.[3] Nucleobases and their alkylated adducts are NHC precursors, and their ability to form ylides[4−7] has enabled their use as biomarkers in certain types of cancer.[8] Notably, only a few examples of NHCs stabilized by transition metals derived from nucleobases have been described.[9−16] For purines, NHC formation employs oxidative addition of the corresponding halogenated nucleobases[9−11] or cyclometalation supported by a chelating unit tether.[9] However, these procedures are restricted to unnatural adducts of adenine and to caffeine and have not been extended to nucleosides. The difficulty of doing so relies mostly on finding a suitable protection/deprotection methodology. While this is a common practice in nucleoside chemistry,[6,10] it cannot be easily transferred to metallated nucleosides. This is due to the harsh conditions employed for deprotection, which compromise the integrity of the compounds in two fundamental ways: the stability of the M−C bond and of the glycosidic bond.[11]

The use of nucleobase derivatives as therapeutic agents suggests that the combination with a metal complex may provide a wide array of pharmaceutical applications, particularly when bound to the metal center as NHC. Metal−NHC systems have been employed as anticancer agents, and one of the main challenges at present is to develop compounds capable of targeting cancerous cells while being nontoxic to healthy ones.[12] For instance, cisplatin is used in more than 50% treatments of cancer patients[13,14] but it presents major drawbacks, such as poor selectivity, intrinsic or acquired resistance,[15] and severe side effects.[14] A suitable approach to overcome this problem is to increase selectivity by utilizing ligands derived from biomolecules that are able to induce selectivity by molecular recognition. As such, complexes that contain tailor-made ligands forming metal bioconjugates could facilitate targeting cancerous cells and achieve a higher selectivity. For purines, coordination to a metal as NHC via C-8 (Scheme 1) provides a connectivity that enables base-pairing interactions, as all sites involved in Watson−Crick base pairing remain intact. This feature constitutes an excellent tool for the development of metal complexes capable of performing targeted molecular recognition.[16−18] With this feature in mind, we considered the development of novel metallo-anticancer agents based on NHCs derived from nucleobases. Herein, we report the synthesis of palladium(II) and platinum(II) NHC complexes based on guanosine. These represent the first examples of deprotected nucleoside NHC complexes that enable base-pairing recognition. As a preliminary evaluation of their antiproliferative ability, we examine their cytotoxic activity against four human cell lines, namely, HEK293, HeLa, PC3, and U251 cells.

RESULTS AND DISCUSSION

The synthesis employs oxidative addition of brominated nucleosides to platinum(0) and palladium(0). Accordingly, the reaction of 2′,3′,5′-triacetyl-8-bromoguanosine[19] (**I**) with $M(PPh_3)_4$ in refluxing toluene (M = Pd, Pt) affords the corresponding guanosine complexes **1a** and **1b** in good yields (Scheme 1). Protection of the hydroxyl groups is required, as the reaction with unprotected 8-bromo-guanosine leads to a mixture of compounds. Complexes **1a** and **1b** can be protonated under acidic conditions at room temperature,

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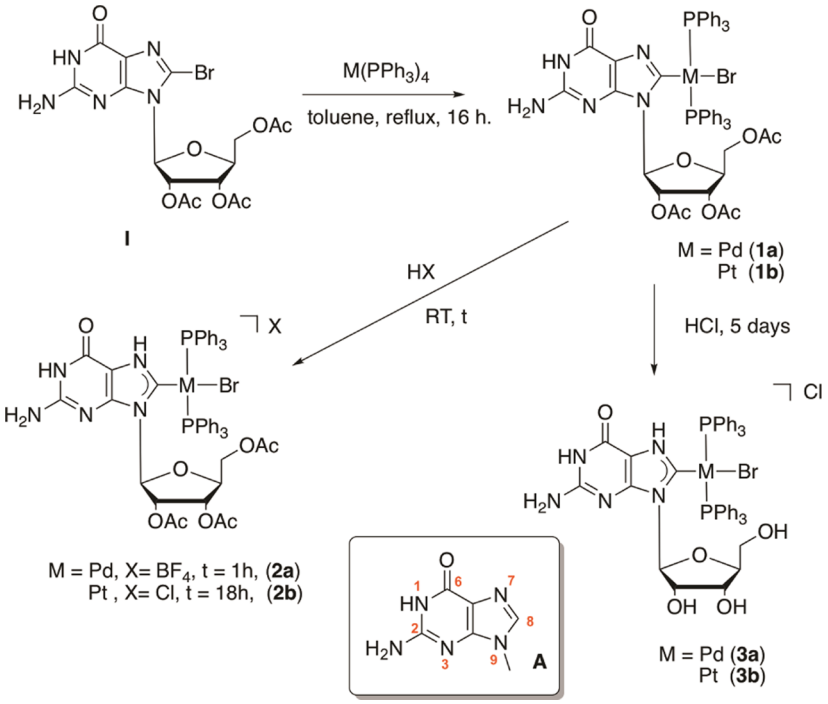
Scheme 1. Synthesis of Guanosine Complexes 1−3 and Numbering Scheme for Purine Nucleobases



affording the corresponding protic NHCs **2a** and **2b**. For **2a**, protonation is induced in methanolic solutions of **1a** with aqueous HBF_4. Earlier attempts to synthesize the corresponding platinum compound from **1b** using HBF_4 were unsuccessful. Thus, compound **2b** was synthesized using ethanolic solutions of HCl. The synthesis of the BF_4 (**2b-BF_4**) derivative can also be achieved using ethanolic solutions of aqueous HBF_4 but in moderate yields. Under acidic conditions similar to those of deprotection but for longer reaction times, compounds **1a** and **1b** undergo deprotection of the hydroxyl groups of the ribose. This deprotection methodology induces concomitant protonation of the nitrogen atom N7 of the guanosine ligand, affording in one step the formation of the protic and deprotected NHCs **3a** and **3b**. Notably, this process does not affect the integrity of the M−C bond. Complexes **1**−**3** are stable under air and moisture for prolonged periods of time. When a solution of **1b** in $CDCl_3$ was kept under air for several weeks, no decomposition was observed by ^{1}H NMR spectroscopy. Complexes **1**−**3** were characterized by NMR spectroscopy in dimethyl sulfoxide-d_6 (DMSO-d_6). As a general trend, in the ^{1}H spectra, the H1′ doublet of the ribose ring undergoes a downfield shift of 0.4−0.9 ppm, with respect to **I**, for all complexes. For compounds **2** and **3**, the N7−H resonates at around 13 ppm, irrespectively of the nature of metal, while the singlet corresponding to the N1−H undergoes an upfield shift of ca. 1 ppm upon protonation. In the $^{13}C\{^1H\}$ NMR spectra, the ribose ring gives rise to five resonances between δ 60 and 90 ppm for all compounds. For complexes **1**, the metallated C-8 is observed at δ 150.2 (**1a**) and 139.9 ppm (**1b**). Upon protonation, the ylidene derivatives **2** and **3** show some differences in $^{13}C\{^1H\}$ NMR spectra, specifically a downfield shift of C-8, (cf. δ 166.9 ppm (**2a**); δ is 153.0 ppm (**2b**)). The $^{31}P\{^1H\}$ NMR spectra present two singlets[20] at around 21−22.00 ppm for palladium complexes and 17−19.00 for platinum complexes. For compounds **1b**, **2b**, and **3**, the $^{31}P\{^1H\}$ spectra show the presence of a minor compound (presumably the enol-tautomer) that we were unable to identify. In ^{1}H NMR, the signals are mostly overlapped (e.g., **2b**) or not detected (**1b**), whereas for $^{13}C\{^1H\}$, the minor product is not observed. For **1b**, the ratio between the minor and the major compound varies when changing from CD_2Cl_2 to DMSO-d_6, further supporting the presence of a tautomeric form of the ligand. Of note, all compounds were characterized by microanalysis to further confirm their purity. Guanine derivatives can aggregate via base-pairing. As an example, aggregation of compound **1a** was monitored by ^{1}H NMR, evidencing that the NH and the NH_2 groups undergo a slight high-field shift (Supporting Information, SI) upon dilution. Aggregation measurements for these complexes are currently being carried out via NMR and will be reported in due course.

Crystals of **2a** were obtained by slow evaporation of a saturated chloroform solution, allowing their characterization by single-crystal X-ray analysis (Figure 1). The crystal structure indicates a trans-orientation for the two phosphines. The Pd−C-8 bond length is 1.981(8) Å, which correlates well with related N-heterocyclic carbene compounds of palladium.[21,22] The sugar adopts a puckered conformation, with the C2 turned out of the plane formed by C1′−O4′−C4′ by 109.8(5)°, following the trend found for purine nucleosides with bulky substituents at the C-8 (C2′ endo).[23−26] The torsion angle around the glycosidic bond (χ, defined by O4′−C1′−N9−C4) is 77.5(9)°, corresponding to a synclinal orientation.[24,27] It reflects a distortion of the ring to accommodate the acetate groups and minimize steric crowding imposed by the phosphines.

The cytotoxicity of the compounds, including the ligand precursor **I**, was tested at concentrations ranging between 0.1 and 80 μM in human embryonic kidney (HEK293) cells, prostate cancer (PC3) cells, cervical cancer (HeLa) cells, and glioblastoma (U251) cells for 48 h. Cytotoxicity was evaluated by the ability of cells to metabolize 3-(4,5-dimethylthiazol-2-

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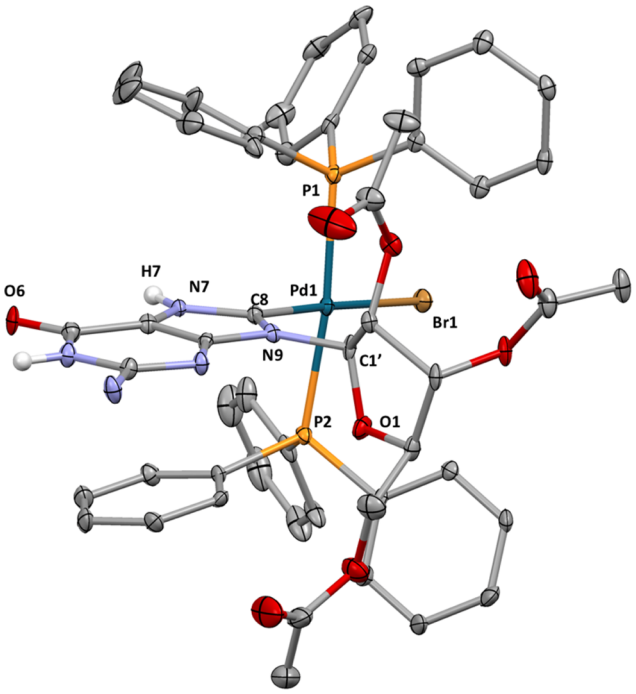

Figure 1. Molecular structure of the cation of complex **2a** (nonrelevant H atoms, solvent molecules and counter ion BF_4 are omitted for clarity, 50% probability ellipsoids).

yl)-2,5-diphenyltetrazolium bromide (MTT), as described elsewhere.[28] Accordingly, healthy human embryonic kidney cells, HEK293, were not sensitive to any of the compounds (cf. SI). For cancerous cell lines PC3 and HeLa, compounds **1**–**3** showed no significant antiproliferative activity. By contrast, glioblastoma U251 cells revealed sensitivity to compounds **2a**, **2b**, and **3a** (Figure 2). Palladium NHC compounds **2a** and **3a** show a higher antiproliferative activity than their corresponding platinum derivative **2b**.

These compounds induce a significant antiproliferative activity when compared with cisplatin for glioblastoma U251. The antiproliferative activity of compound **1a** presents a large variability between experiments and thus is not found statistically significant. This is probably a result of its low solubility, as **1a** precipitates at higher concentrations upon addition to liquid cell culture medium. In contrast, the measurements for **2a** and **3a** are consistent between experiments. For example, the antiproliferative activity of compound **3a** reaches ca. 50% for a range of concentrations between 20 and 80 μM. For platinum complexes, the higher cytotoxic activity is observed for compound **2b**, the acetate-protected platinum NHC. Compound **2b** induces a significant decrease in cancer cell growth at a concentration as low as 0.1 μM, but in general, the maximum percentage of decrease was less significant than that of compounds **2a** and **3a**. The ligand precursor **I**, the brominated and protected guanosine, does not show antiproliferative activity.

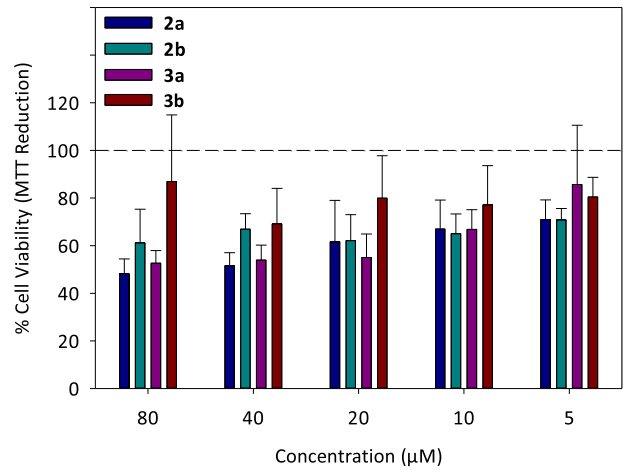

Figure 2. Viability of U251 cells after incubation with compounds **2a**,**b** and **3a**,**b** for 48 h, assessed by evaluating their ability to metabolize MTT.

CONCLUSIONS

In summary, we have disclosed a new methodology for the synthesis of N-heterocyclic carbenes derived from guanosine with palladium(II) and platinum(II) precursors. For the reaction to be effective, the guanosine derivative requires protection of its hydroxyl groups with acetate. This constitutes the first example of a purine NHC derived from a nucleoside. The deprotected NHC can subsequently be obtained under acidic conditions. Antiproliferative measurements with a number of cell lines show that the compounds are nontoxic for healthy cells HEK293. Compounds **2a**, **2b**, and **3a** are active cytotoxic agents for glioblastoma cell line U251 and show a significant antiproliferative activity when compared with cisplatin. These are promising results, as glioblastoma multiform is a very aggressive form of brain cancer in adults.[29] Studies on other glioblastoma cell lines and examination of the mechanism of action of these compounds are currently being pursued.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02387.

Crystallographic data (CIF)

Experimental procedures, NMR and mass spectra, and cytotoxic studies (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Professor Ernesto Carmona on the occasion of his 70th birthday.

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