Metal-Ion-Binding Analogs of Ribonucleosides: Preparation and Formation of Ternary Pd^{2+} and Hg^{2+} Complexes with Natural Pyrimidine Nucleosides

by **Oleg Golubev**, **Tuomas Lönnberg**, and **Harri Lönnberg***

University of Turku, Department of Chemistry, FI-20014 Turku
(phone: +358-2-3336770; fax: +358-2-3336700; e-mail: harlon@utu.fi)

Four metal-ion-binding nucleosides, *viz*. 2,6-bis(1-methylhydrazinyl)-9-(β-D-ribofuranosyl)-9*H*-purine (**2a**) and its *N*-acetylated derivative, **2b**, 2,4-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-(β-D-ribofuranosyl)pyrimidine (**3**), and 2,4-bis(1-methylhydrazinyl)-5-(β-D-ribofuranosyl)pyrimidine (**4**) have been synthesized. The ability of these nucleosides and the previously prepared 2,6-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-9-(β-D-ribofuranosyl)-9*H*-purine to form Pd^{2+}- and Hg^{2+}-mediated complexes with uridine has been studied by ^{1}H-NMR spectroscopy. To obtain additional support for the interpretation of the NMR data, comparative measurements on the ternary-complex formation between pyridine-2,6-dicarboxamide (**5**), pyrimidine nucleosides, and K_2PdCl_4 were carried out.

Introduction. – Metal-ion-mediated base-pairing of nucleic acids has attracted considerable attention during the past decade [1–3]. Interest in this area originates from the desire to expand the genetic code by artificial base pairs [4–6], to create predesigned molecular architecture by metal-ion-mediated inter- or intrastrand cross-links [7–14], or to convert double-stranded DNA to a nano-scale wire by making use of consecutive Hg^{II}- or Ag^{I}-ion mediated TT [15], CC [16], T(1-deazaA) [17], Im-Im [18], or 5-substituted UU base pairs [19]. Such duplexes have also found applications as sensitive detectors of metal ions [20–27]. An interesting example of cooperative action of metal-ion coordination and small-molecule binding is offered by cross-linking of salen moieties on opposite strands by ethylenediamine in the presence of Cu^{2+} [28–30]. The otherwise unstable diimine cross-link is stabilized by square-planar binding of Cu^{2+} to the imine N-atoms and salen OH functions.

Formation of T-Hg^{2+}-T and C-Ag^{+}-C base pairs, apart all the metal-ion-mediated hybridizations mentioned above, depend on the presence of a modified nucleobase in both strands engaged in the duplex formation. The data on hybridization of metal-ion-binding oligonucleotide analogs with natural nucleic acid sequences are scanty. It has been shown that metal-ion-induced changes in the secondary structure of an oligonucleotide probe affect the strength of binding to an unmodified DNA target [31], but discrimination between the four natural nucleobases by metal-ion-binding surrogate bases is still an unsolved problem. Tentatively, high-affinity binding might be achieved by coordination of N(1) of purines and N(3) of pyrimidines to a soft metal ion carried by an artificial nucleobase, while selectivity could be obtained by a proper combination of destabilizing steric and stabilizing H-bonding interactions. Some recent observations lend evidence for the feasibility of this approach. It has been shown that 2,6-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-9*H*-purine riboside (**1**) at the 3′-terminus of a 6-

mer 2′-*O*-methyl-RNA oligonucleotide is markedly stabilizing in the presence of Cu^{2+}, exhibiting some preference for U and G [32]. In an intrachain position, the stabilizing influence is less prominent. A purine base bearing two pyrazole rings possibly is so bulky that it tends to distort the normal double helical structure upon formation of a Cu^{2+}-mediated base pair. A smaller pyrimidine-derived analog might be more appropriate for the purpose. We now report on the synthesis of two such nucleosides, 2,4-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-(β-D-ribofuranosyl)pyrimidine (**3**) and 2,4-bis(1-methylhydrazinyl)-5-(β-D-ribofuranosyl)pyrimidine (**4**). In addition, 2,6-bis(1-methylhydrazinyl)-9-(β-D-ribofuranosyl)-9*H*-purine (**2a**) and its *N*-acetylated derivative, **2b** (*Fig. 1*), have been prepared as less-bulky analogs of **1**. The ability of the metal-ion-binding nucleosides **1**–**4** to form Pd^{2+}- and Hg^{2+}-mediated complexes with uridine has been studied by ^{1}H-NMR spectroscopy. As a simplified model system, ternary-complex formation between pyridine-2,6-dicarboxamide (**5**), pyrimidine nucleosides, and K_2PdCl_4 has been studied to obtain support for the interpretation of the NMR data.

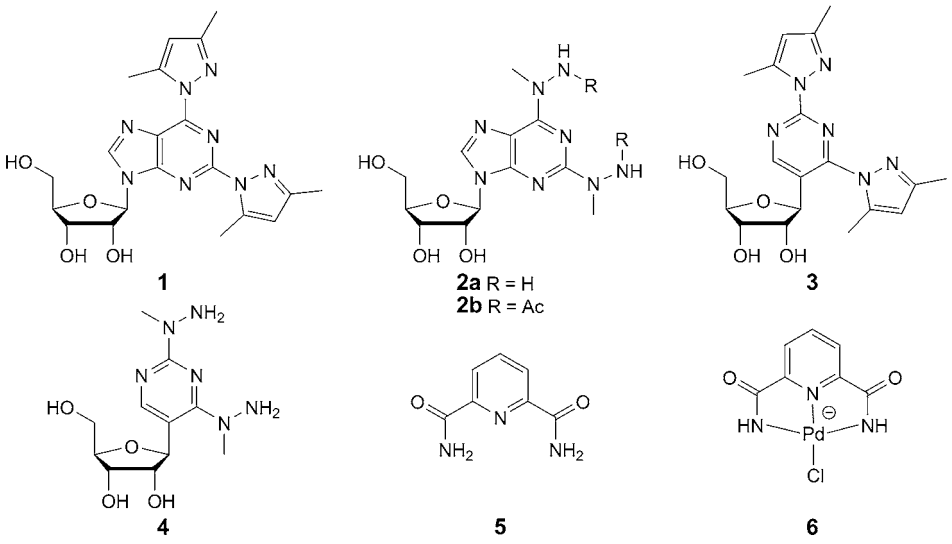
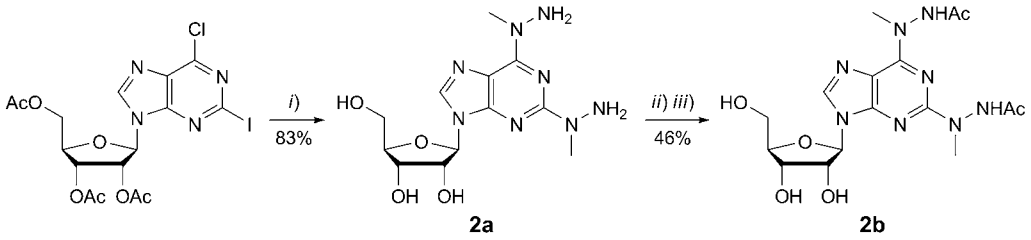


Fig. 1. *Structures of the metal-ion-binding nucleosides* **1**–**4**, *and the corresponding model compounds* **5** *and* **6**

Results and Discussion. – *Syntheses of the Metal-Ion-Binding Nucleosides.* Preparation of 2,6-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-9-(β-D-ribofuranosyl)-9*H*-purine (**1**) has been described in [33]. 2,6-Bis(1-methylhydrazinyl)-9-(β-D-ribofuranosyl)-9*H*-purine (**2a**) and its *N*-acetylated derivative, **2b**, were prepared from commercially available 2′,3′,5′-tri-*O*-acetyl-6-chloro-2-iodo-9-(β-D-ribofuranosyl)-9*H*-purine as outlined in *Scheme 1*. Accordingly, treatment of the starting material with aqueous $MeNHNH_2$ for 2 d and subsequent crystallization from ^{i}PrOH gave **2a** as a white powder. To obtain **2b**, compound **2a** was first peracetylated, and the *O*-Ac groups were

Scheme 1. *Preparation of 2,6-Bis(1-methylhydrazinyl)-9-(β-*D*-ribofuranosyl)-9*H*-purine* (**2a**) *and Its* N*-Acetylated Derivative*, **2b**

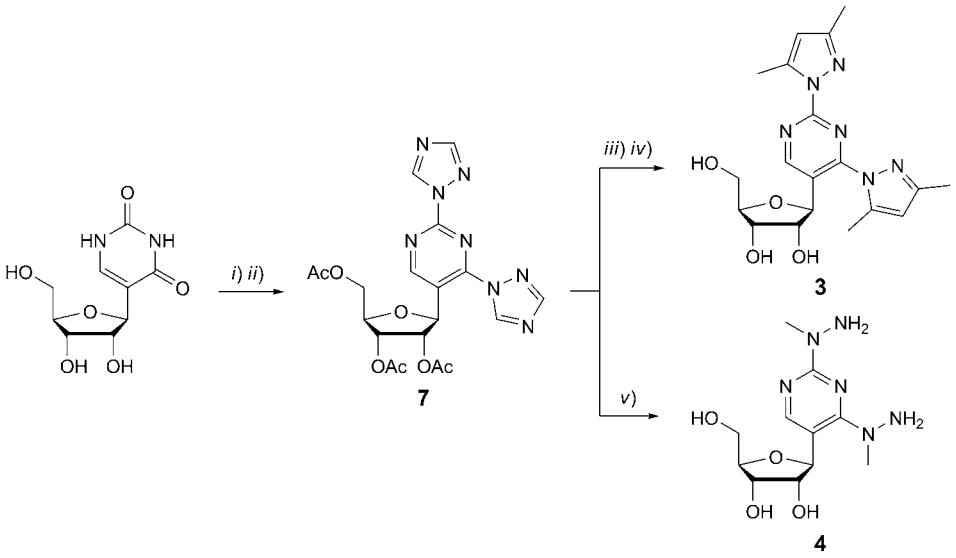


i) $MeNHNH_2$ (aq.). *ii*) Ac_2O, pyridine. *iii*) MeONa, MeOH.

then removed by MeONa-catalyzed transesterification in MeOH. The crude product was purified by reversed-phase HPLC.

2,4-Bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-(*β*-D-ribofuranosyl)pyrimidine (**3**) and 2,4-bis(1-methylhydrazinyl)-5-(*β*-D-ribofuranosyl)pyrimidine (**4**) were synthesized by converting 2′,3′,5′-tri-*O*-acetylated pseudouridine first to 2,4-di(1*H*-1,2,4-triazol-1-yl)-9-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)pyrimidine (**7**) by $POCl_3$-promoted replacement of the oxo groups with 1,2,4-triazole (*Scheme 2*) [34]. Replacement of the 1,2,4-triazol-1-yl groups with NH_2NH_2 and subsequent treatment with pentane-2,4-dione [35] gave **3** in moderate yield. Reaction of **7** with $MeNHNH_2$, in turn, yielded the 2,4-

Scheme 2. *Preparation of 2,4-Bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-(β-*D*-ribofuranosyl)pyrimidine* (**3**) *and 2,4-Bis(1-methylhydrazinyl)-5-(β-*D*-ribofuranosyl)pyrimidine* (**4**)



i) Ac_2O, Pyridine. *ii*) $POCl_3$, 1,2,4-triazole, Et_3N, MeCN. *iii*) $NH_2NH_2 \cdot H_2O$. *iv*) Pentane-2,4-dione, CF_3COOH, 24 h. *v*) $MeNHNH_2$.

bis(1-methylhydrazinyl) analog **4**. The sugar Ac groups were removed during the treatment with NH_2NH_2 or $MeNHNH_2$.

Ternary Pd^{2+} Complexes of Pyridine-2,6-dicarboxamide with Uridine and Cytidine. The ability of pyridine-2,6-dicarboxamide (**5**) to form ternary Pd^{2+} complexes with uridine and cytidine was first studied as a simplified model system of the ternary-complex formation of the metal-binding nucleosides **1**–**4**. To elucidate the binding mode of Pd^{2+}, **5** was treated with 1 equiv. of K_2PdCl_4 in aqueous solution, and the precipitated complex **6** was characterized by NMR spectroscopy and mass spectrometry. According to negative-ion-mode HR-ESI-MS, Pd^{2+} was bound to **5** as a monochlorido complex by displacement of two H-atoms (*m/z* 303.9085; calc. for $C_7H_5ClN_3O_2Pd$: 303.9105). Replacement of two of the amido H-atoms was verified by ^{1}H-NMR recordings in (D_6)DMSO: the two H-atom resonances at 8.17 and 8.86 ppm were replaced by a single 2 H resonance at 6.37 ppm. At the same time, the *multiplet* of H–C(2), H–C(3), and H–C(4) at 8.11–8.18 was split into two separate signals: a *triplet* at 8.30 ($J = 7.8$) referring to H–C(4), and a *doublet* at 7.75 ($J = 7.8$) referring to H–C(3) and H–C(5).

Complexing of **5** with K_2PdCl_4 was then studied in D_2O at pH 7.2 (0.12M phosphate buffer). The signals of the amido H-atoms could not be detected, owing to rapid deuteration, but the presence of a 1 H-atom *triplet* at 8.12 ($J = 7.7$) and a 2 H *doublet* at 7.59 ($J = 7.7$) evidenced quantitative complexing of **5** with Pd^{II} even at the concentration of 0.25 mM of both species, which was the lowest concentration used in the subsequent recordings. In other words, the equilibrium of *Eqn. 1*,

$$LH_2 + PdCl_4^{2-} \rightleftharpoons LPdCl^- + 3\ Cl^- + 2\ H^+ \quad (1)$$

where LH_2 stands for **5**, always lies far on the right hand side. Addition of uridine to the system resulted in formation of a new set of signals. The H–C(5) resonance of uridine was shifted from 5.81 ($J = 8.1$) to 5.74 ppm ($J = 7.7$), the H–C(6) resonance from 7.78 ($J = 8.1$) to 7.67 ppm ($J = 7.7$ Hz), and the H–C(1′) resonance from 5.83 ($J = 4.6$) to 5.86 ppm ($J = 4.4$). The H–C(4) *triplet* of **6** underwent, in turn, a downfield shift from 8.12 to 8.18 ppm, the H–C(3) and H–C(5) *doublet* remaining almost unchanged. *Fig. 2* shows the signals attributed to different components of the reaction mixture at 0.5 mM concentration. In all likelihood, a ternary complex was formed by replacement of the chlorido ligand of **6** with *N(3)*-deprotonated uridine, as shown previously for binding of uridine to the tridentate $[Pd(dien)Cl]^+$ and $[Pd(terpy)Cl]^+$ complexes [36][37]. Upon increasing the concentration of all the components (uridine, **5**, and K_2PdCl_4), but keeping the 1:1:1 molar ratio (pH 7.2), the intensity of the new signals was gradually increased compared to the intensity of the signals of **6** and uridine. At 0.3 mM concentration of the components, the concentrations of complexed and free uridine were equal. Formation of the ternary complex can be described by *Eqn. 2*, and the equilibrium constant, K_{Urd}, for this reaction by *Eqn. 3*.

$$Urd^- + LPdCl^- \rightleftharpoons LPdUrd^- + Cl^- \quad (2)$$

$$K_{Urd} = [LPdUrd^-][Cl^-](K_a + [H^+])/\{K_a\,[LPdCl^-][Urd_{free}]\} \quad (3)$$

HUrd denotes here neutral uridine, Urd^- *N(3)*-deprotonated uridine monoanion, and Urd_{free} the concentration of uncomplexed uridine, regardless of its ionic form. K_a is the acidity constant of HUrd. At the 0.30 mM concentration of uridine, **5**, and K_2PdCl_4, where 50% of uridine is engaged in the ternary complex, $[LPdUrd^-] = [Urd_{free}] = [LPdCl^-] = 0.15$ mM and $[Cl^-] = 1.05$ mM. Accordingly, $K_{Urd} = 7.0 \times (K_a + [H^+]) \times K_a^{-1} = 1400$ (pK_a value of uridine is 9.5 in D_2O [36]), and log K_{Urd} for binding of the *N(3)*-deprotonated uridine monoanion to the Pd^{2+} complex of pyridine-2,6-dicarboxamide, hence, is 3.1.

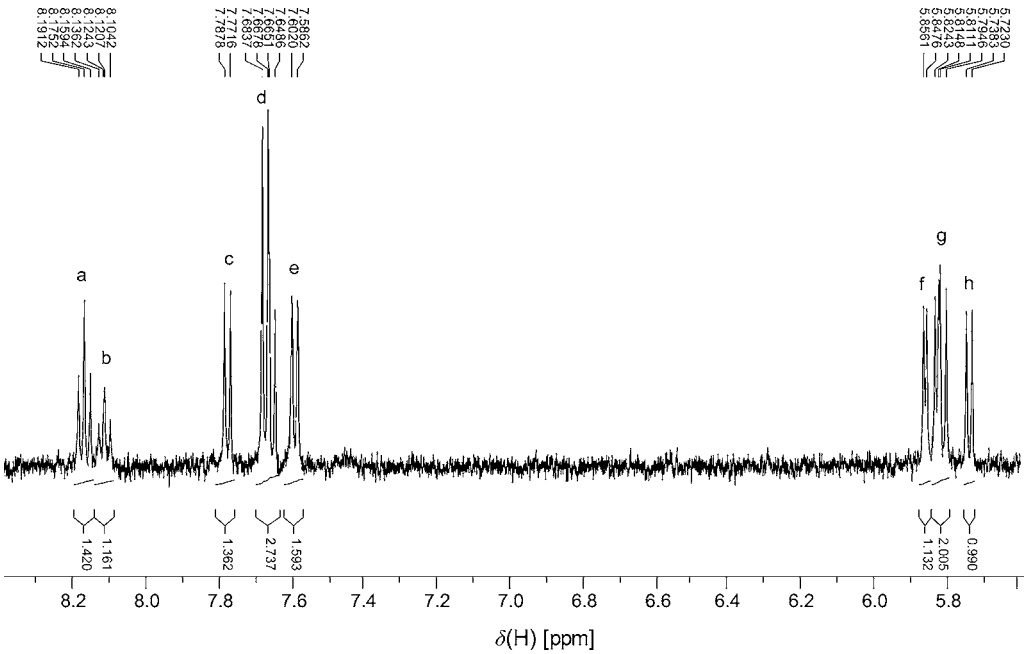


Fig. 2. *Part of the ^{1}H-NMR spectrum of the mixture of uridine, pyridine-2,6-dicarboxamide* (**5**)*, and K_2PdCl_4 in D_2O* (pH 7.2, 0.12M phosphate buffer). The concentration of each component is 0.50 mM; L^{2-} refers to the dianion of **5**. a: H–C(4) of **5** in $LPdUrd^-$, b: H–C(4) of **5** in $LPdCl^-$, c: H–C(6) of Urd^- in $LPdUrd^-$, d: H–C(3) and H–C(5) of **5** in $LPdCl^-$ and $LPdUrd^-$, e: H–C(6) of HUrd, f: H–C(1′) of Urd^- in $LPdUrd^-$, g: H–C(1′) and H–C(5) of HUrd, and h: H–C(5) of Urd^- in $LPdUrd^-$.

The situation remained rather similar on using cytidine as the nucleosidic constituent of the ternary complex, although the affinity to cytidine was somewhat lower than that to uridine: 50% engagement of **5** and cytidine in the complex was achieved at 0.5-mM concentration of **5**, cytidine, and K_2PdCl_4. Upon complex formation, the H–C(5) resonance of cytidine was shifted from 5.96 ($J = 7.6$) to 6.05 ppm ($J = 7.5$) and the H–C(6) resonance from 7.75 to 7.86 ppm (*Fig. 3*). These spectral changes were accompanied by the shift of the H–C(3) and H–C(5) *doublet* of **5** from 7.59 ($J = 7.6$) to 7.70 ppm ($J = 7.8$). At higher concentrations, additional signals at 6.9 – 7.4 and 5.5 – 5.6, however, appeared. These most likely refer to formation of a

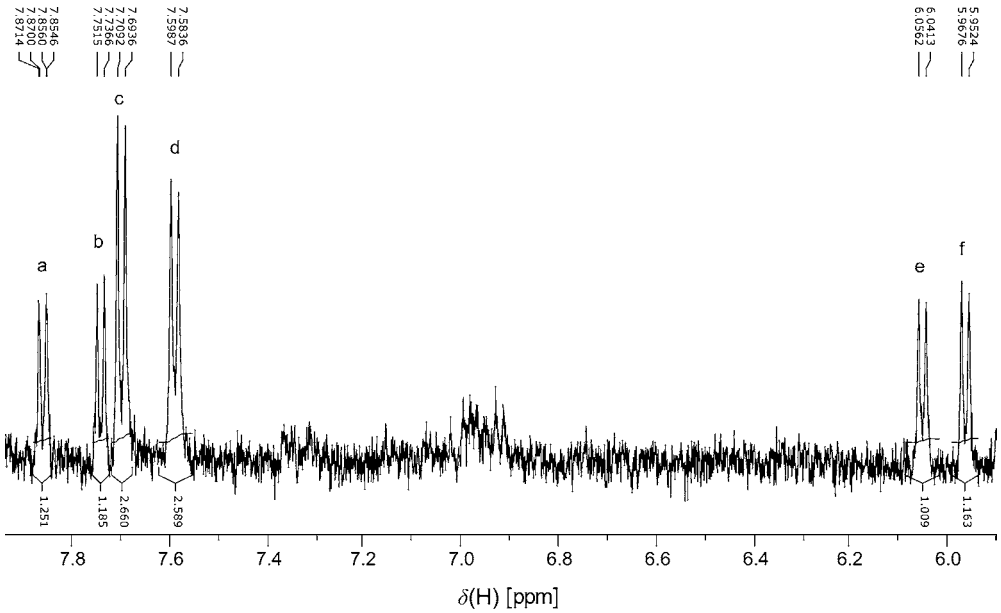


Fig. 3. *Part of the ^{1}H-NMR spectrum of the mixture of cytidine, pyridine-2,6-dicarboxamide* (**5**)*, and K_2PdCl_4 in D_2O* (pH 7.2, 0.12M phosphate buffer). The concentration of each component is 0.5 mM; L^{2-} refers to the dianion of **5**. a: H–C(6) of Ctd in LPdCtd, b: H–C(6) of Ctd, c: H–C(3) and H–C(5) of **5** in LPdCtd, d: H–C(3) and H–C(5) of **5** in LPdCl, e: H–C(5) of Ctd in LPdCtd and f: H–C(5) of Ctd.

2 : 2 : 1 complex obtained by replacement of one of the amino group H-atoms of cytidine with another Pd^{II} complex of **5**. The occurrence of this kind of binding mode has been earlier shown with $[Pd(dien)]^{2+}$ binding to 1-methylcytosine [37].

In contrast to uridine, cytidine is engaged in the ternary complex as a neutral species. Accordingly, the complex formation and the equilibrium constant, K_{Ctd}, for this reaction can be expressed by *Eqns. 4* and *5*, respectively, as long as formation of a 1 : 1 : 1 ternary complex is concerned.

$$LPdCl^- + Ctd \rightleftharpoons LPdCtd + Cl^- \quad (4)$$

$$K_{Ctd} = [LPdCtd][Cl^-]/[LPdCl^-][Ctd] \quad (5)$$

At the equimolar 0.50-mM concentration of the participating compounds where half of cytidine is complexed, $[L^1PdCtd] = [Ctd_{free}] = [L^1PdCl^-] = 0.25$ mM, $[Cl^-] = 1.75$ mM, and $K_{Ctd} = 7$ ($\log K_{Ctd} = 0.8$). In other words, the affinity of *N(3)*-deprotonated uridine to **6** is 200-fold compared to that of cytidine N(3). The selectivity is somewhat lower than the 800-fold difference reported for the stability of the complexes of 1-methyluracil monoanion and 1-methylcytosine with the $[Pd(dien)Cl]^+$, or the 6300-fold difference of the similar complexes $[Pd(terpy)Cl]^+$ [37].

Fig. 4 shows the PM6-minimized structures for the complexes of pyridine-2,6-dicarboxamide with Pd^{2+} with uridine and cytidine. Evidently, the C=O O-atoms of the

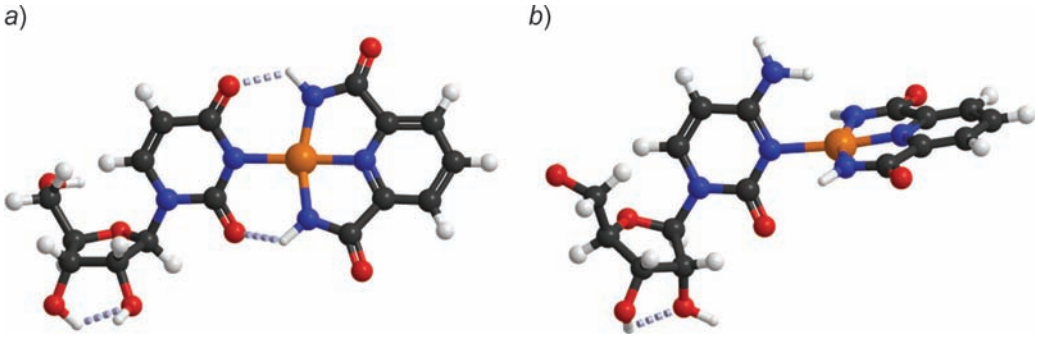


Fig. 4. *PM6-Minimized structures for the complexes of* N(3)*-deprotonated uridine* (*a*) *and neutral cytidine* (*b*) *with pyridine-2,6-dicarboxamido* Pd^{2+}

uracil base are H-bonded to the amido N-atoms of the pyridine-2,6-dicarboxamido ligand, which forces the uracil and pyridine rings in almost the same plane. MM2 Calculations suggest the dihedral angle $C(2)_{Ura}$–$N(3)_{Ura}$–Pd–$N_{carboxamide}$ to be *ca.* 20 degrees (*Fig. 5*). In striking contrast, with cytidine complex the planes of the two ligands are almost perpendicular, the co-planar orientation being strongly disfavored by steric repulsion between the 2-O and 4-NH_2 substituents of the cytosine ring and the carboxamido groups, respectively.

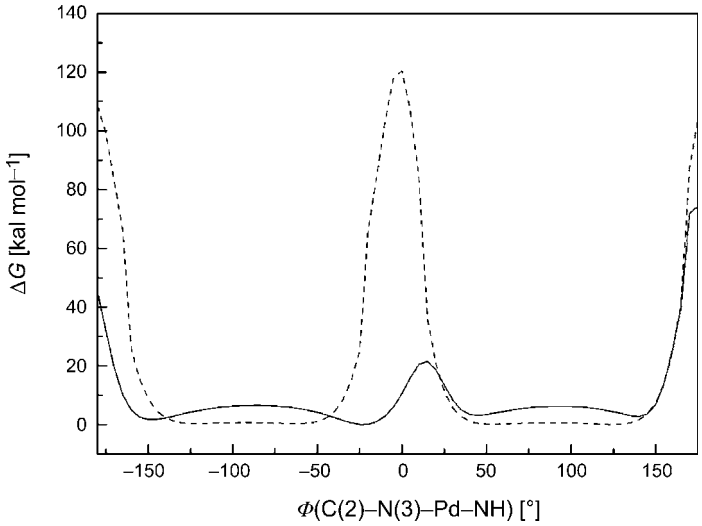


Fig. 5. *Free-energy change for the rotation around the Pd–N(3) bond of the complexes of uridine* (solid line) *and cytidine* (dotted line) *with pyridine-2,6-dicarboxamido–*Pd^{2+}

Ternary Pd^{2+} *Complexes of Metal-Ion-Binding Nucleosides* **1**–**4** *with Uridine and Cytidine*. Our previous ^{1}H-NMR studies [33] have shown that 2,6-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-9-(*β*-D-ribofuranosyl)-9*H*-purine (**1**) forms a very stable ternary Pd^{2+}

complex with uridine. At the lowest concentration where the NMR recordings could be performed, *viz.* [**1**] = [Urd] = K_2PdCl_4 = 0.06 mM, 80% of uridine was still engaged in the ternary complex at pH 7.3. Assuming that formation of the complex $[Pd(\mathbf{1})Cl]^+$ is quantatitative (as above with **5**), the equilibrium concentrations are: $[(\mathbf{1})PdUrd^-]$ = 0.048 mM, $[Urd_{free}] = [(\mathbf{1})PdCl^-]$ = 0.012 mM, and $[Cl^-]$ = 0.228 mM. The equilibrium constant for the formation of the ternary complex $[Pd(\mathbf{1})Urd]^+$ (*cf. Eqn. 3*) is $K(\mathbf{1})_{Urd}$ = 15000 ($\log K(\mathbf{1})_{Urd} = 4.2$), *i.e.*, one order of magnitude higher than the value of the corresponding uridine complex of **5**.

Somewhat unexpectedly, the Pd^{2+} complex of 2,6-bis(1-methylhydrazinyl)-9-(β-D-ribofuranosyl)-9*H*-purine (**2a**) appears to exhibit a much lower tendency to bind uridine. Even at an equimolar 3.2 mM concentration of **2a**, uridine, and K_2PdCl_4, only 48% of uridine was observed to be complexed (*Fig. 6*). Assuming again that the binding of Pd^{2+} to **2a** is virtually quantitative, the equilibrium constant for the formation of the ternary complex (*cf. Eqn. 3*) is $K(\mathbf{2a})_{Urd}$ = 1200 ($\log K(\mathbf{2a})_{Urd} = 3.1$), *i.e.*, almost equal to the stability constant of the corresponding complex of pyridine-2,6-dicarboxamide. The *N*-acetylated analog **2b** did not show any sign of ternary-complex formation.

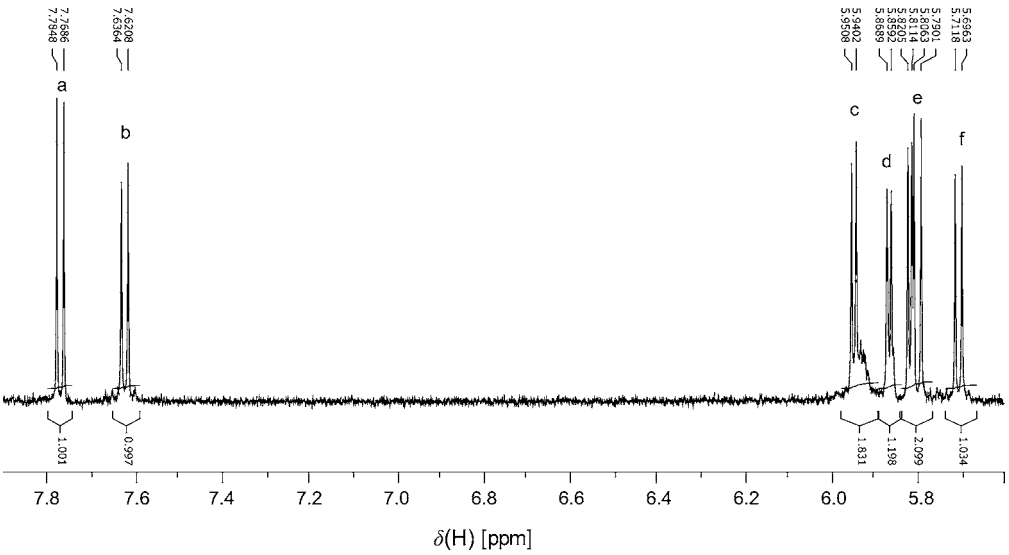


Fig. 6. *Part of the ^{1}H-NMR spectrum of the mixture of uridine, 2,6-bis(1-methylhydrazinyl)-9-(β-D-ribofuranosyl)-9*H*-purine* (**2a**)*, and K_2PdCl_4 in D_2O* (pH 7.2, 0.12M phosphate buffer). The concentration of each component is 3.2 mM. a: H–C(6) of HUrd, b: H–C(6) of Urd^- in (**2a**)$PdUrd^+$, c: H–C(1′) of **2a** in (**2a**)$PdCl^+$ and (**2a**)$PdUrd^+$, d: H–C(1′) of Urd^- in (**2a**)$PdUrd^+$, e: H–C(1′) and H–C(5) of HUrd, and f: H–C(5) of Urd^- in (**2a**)$PdUrd^+$.

The NMR studies on formation of a mixed-ligand Pd^{2+} complex between pseudouridine derivative **3** and uridine were severely disturbed by precipitation. Although **3** and uridine were at 5-mM concentration soluble into the phosphate buffer in D_2O, addition of K_2PdCl_4 in 0.1-equiv. portions into their equimolar mixture resulted in diminution of the signals both nucleosides without appearance of any clearcut signals

attributable to the ternary complex. The ratio of the signal intensities of **3** and uridine remained constant, suggesting that the precipitate contained the two nucleosides in 1:1 ratio. Comparison of the signal intensities to those of EtOH, used as an internal standard, revealed that *ca.* 25% of **3** and uridine had precipitated (assumingly as a ternary complex) when the total concentrations of **3**, uridine, and K_2PdCl_4 were 3.6, 3.6, and 1.4 mM, respectively. Accordingly, formation of the ternary complex evidently was much weaker than with **1**, *ca.* of the order of 100. With the 2,4-bis(1-methylhydrazinyl)-substituted pseudouridine (**4**), the precipitation was even more extensive preventing acquisition of any quantitative data.

Ternary Hg^{2+} Complexes of Metal-Ion-Binding Nucleosides **1**–**4** *with Uridine and Cytidine.* The ability of metal-ion-binding nucleosides **1**–**4** to form ternary complexes with uridine was additionally studied by using $HgCl_2$ as the source of the central metal ion. For this purpose, $HgCl_2$ was gradually added into a 1:1 mixture of uridine and one of nucleosides **1**–**4** in D_2O (each 5 mM) at pH 7.2 (0.12 mM phosphate buffer). After each addition, a ^{1}H-NMR spectrum of the mixture was recorded. In contrast to Pd^{2+}, the ligand-exchange reactions of Hg^{2+} are fast in NMR time-scale, and, hence, metal-ion binding does not give a new set of signals but only shifts the signals of the ligands. *Fig. 7* displays the changes in chemical shifts of some relevant resonances upon addition of $HgCl_2$ into a mixture of uridine and **1**. On examining the data, one should bear in mind that the total concentrations of the nucleosides decreased with the increasing concentration of $HgCl_2$, owing to the dilution caused by addition of the buffered $HgCl_2$ solution. In spite of this complication, it appears clear that the affinity of $HgCl_2$ to uridine is higher than that to 2,6-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-9-(*β*-D-ribofura-

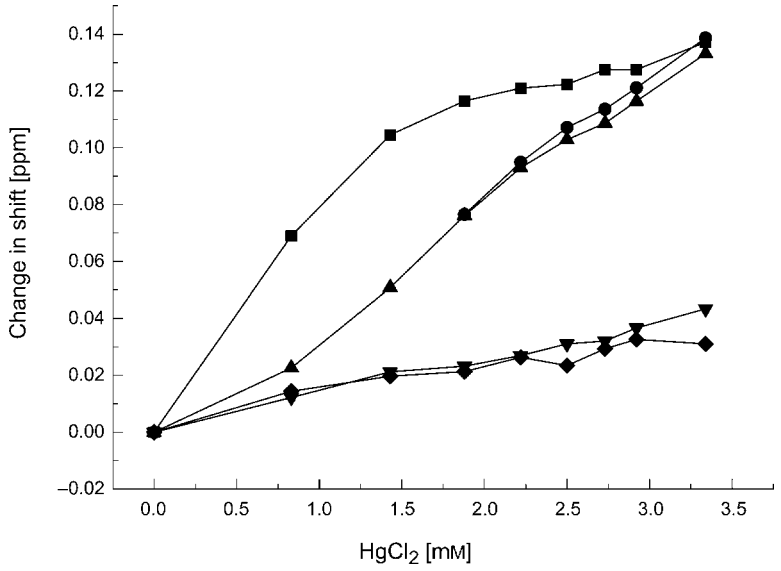


Fig. 7. *Hg^{2+}-Induced shifts on some relevant resonances of uridine and 2,6-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-9*H*-purine riboside* (**1**) *in their equimolar mixture in D_2O* (pH 7.2, 0.12M phosphate buffer). ■: H–C(5) of uridine, ●: H–C(8) of **1**, ▲: H–C(4)(pyrazolyl) of **1**, ▼: H–C(1′) of **1**, ◆: H–C(1′) of uridine.

nosyl)-9*H*-purine: the H–C(5) resonance of uridine is shifted at submillimolar concentrations of $HgCl_2$ more markedly than H–C(8) or pyrazolyl H–C(4) resonances of **1**. The predominant species most likely is Urd_2Hg. When the concentration of $HgCl_2$ reaches that of the nucleosides, both nucleosides exhibit comparable changes in these resonances, suggesting that the mixed complex, [Hg(**1**)Urd]$^+$, predominates. This complex, however, seems to be formed only at mM concentrations of both nucleosides and $HgCl_2$, indicating that the ternary complex is considerably less stable that the corresponding ternary Pd^{2+} complex.

Addition of $HgCl_2$ to an equimolar mixture of **3** and uridine resulted in even smaller shifts on the relevant H-atom resonances, the magnitude of the shifts increasing almost linearly with the metal-ion concentration. Evidently, the complex formation was weaker than with **1**. The situation was similar with **4**, and precipitation prevented recording with 2,6-bis(1-methylhydrazinyl)-9-(β-D-ribofuranosyl)-9*H*-purine (**2a**).

Conclusions. – 2,4-Bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-(β-D-ribofuranosyl)pyrimidine (**3**) has been prepared as a less bulky analog of the previously [33] introduced metal-ion-binding purine nucleoside, 2,6-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-9-(β-D-ribofuranosyl)-9*H*-purine (**1**). This novel pyrimidine nucleoside has been shown to form a mixed-ligand Pd^{2+} complex with uridine, although somewhat less efficiently than its purine analog **1**. The compound, hence, seems to be a viable candidate for construction of oligonucleotide probes exhibiting metal-ion-mediated base pairing. Replacement of the 3,5-dimethyl-1*H*-pyrazol-1-yl with 1-methylhydrazinyl groups did not improve the complex formation of either **1** or **3**. Mixed-ligand Hg^{2+} complexes of **1** and **3** with uridine turned out to be less stable than the corresponding Pd^{2+} complexes.

Experimental Part

General. The preparation of 2,6-bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-9-(β-D-ribofuranosyl)-9*H*-purine has been described in [33]. The solvents were dried over 4-Å molecular sieves. Column chromatography (CC): silica gel *60* (SiO_2; 230–400 mesh, *Fluka*). UV Spectra: *Perkin-Elmer UV/VIS lambda 10*; λ_{max} in nm. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance* 500- or 400-MHz NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *Bruker Daltonics micrOTOF-Q* instrument; in m/z.

*2,6-Bis(1-methylhydrazinyl)-9-(β-D-ribofuranosyl)-9*H*-purine* (**2a**). Commercial 6-chloro-2-iodo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (1 mmol, 0.538 g) was dissolved in $MeNHNH_2/H_2O$ 2:1 (*v*/*v*; 15 ml). The mixture was stirred at r.t. for 48 h and evaporated to dryness under reduced pressure. The solid residue was recrystallized from ^{i}PrOH to yield **2a** (0.83 mmol, 0.282 g, 83%). White powder. UV: 245, 294. ^{1}H-NMR (400 MHz, D_2O): 7.75 (*s*, H–C(8)); 5.75 (*d*, J = 5.5, H–C(1′)); 4.63 (*dd*, J = 5.0, 5.5, H–C(2′)); 4.26 (*dd*, J = 4.7, 5.0, H–C(3′)); 4.03–4.06 (*m*, H–C(4′)); 3.75 (*dd*, J = 3.2, 12.6, H–C(5′)); 3.66 (*dd*, J = 4.9, 12.6, H–C(5″)); 3.25 (*s*, MeN); 3.06 (*s*, MeN). ^{13}C-NMR (100 MHz, D_2O): 158.0 (C(2)); 152.3 (C(4)); 152.0 (C(6)); 137.2 (C(8)); 111.4 (C(5)); 87.7 (C(1′)); 84.6 (C(4′)); 73.1 (C(2′)); 70.2 (C(3′)); 61.3 (C(5′)); 39.8 (MeN); 38.3 (MeN). HR-ESI-MS: 341.1665 ([M + H]$^+$, $C_{12}H_{21}N_8O_4^+$; calc. 341.1686).

*2,6-Bis(2-acetyl-1-methylhydrazinyl)-9-(β-D-ribofuranosyl)-9*H*-purine* (**2b**). Compound **2a** (1 mmol, 0.340 g) was dissolved in a 1:2 (*v*/*v*) mixture (30 ml) of freshly dist. Ac_2O and dry pyridine. The mixture was stirred at r.t. for 24 h, and the volatiles were then removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 ml), washed with sat. aq. $NaHCO_3$ (50 ml), and the org. phase was dried (Na_2SO_4). CC (SiO_2; CH_2Cl_2/MeOH 35:5 (*v*/*v*)) gave 2′,3′,5′-tri-*O*-acetylated **2b** in nearly quantitative yield. HR-ESI-MS: 549.2086 ([M – H]$^-$, $C_{22}H_{29}N_8O_9^-$; calc. 549.2058).

Part of the product obtained (0.18 mmol, 0.100 g) was dissolved in methanolic NH_3 (7M, 2.0 ml), and the mixture was stirred at r.t. for 3 h. The volatiles were removed under reduced pressure, and the residue was purified by HPLC (*LiCroCART® 250–10* column; aq. $AcONH_4$/MeCN 93:7 (*v/v*)). The buffer constituents were removed under reduced pressure to give **2b** (0.09 mmol, 0.036 g, 46%). UV: 232, 261 (sh), 286. ^{1}H-NMR (400 MHz, D_2O): 7.90 (*s*, H–C(8)); 5.87 (*d*, *J* = 5.5, H–C(1′)); 4.81 (*dd*, *J* = 5.0, 5.5, H–C(2′)); 4.36 (*dd*, *J* = 4.7, 5.0, H–C(3′)); 4.09–4.12 (*m*, H–C(4′)); 3.78 (*dd*, *J* = 3.3, 12.5, H–C(5′)); 3.70 (*dd*, *J* = 5.0, 12.5, H–C(5″)); 3.38 (*s*, MeN); 3.24 (*s*, MeN); 2.05 (*s*, MeCO); 2.00 (*s*, MeCO). ^{13}C-NMR (100 MHz, D_2O): 173.7 (C=O); 173.4 (C=O); 158.5 (C(4)); 155.5 (C(2)); 152.6 (C(6)); 139.3 (C(8)); 114.1 (C(5)); 88.4 (C(1′)); 84.9 (C(4′)); 72.7 (C(2′)); 70.5 (C(3′)); 61.5 (C(5′)); 38.2 (MeN); 38.1 (MeN); 20.0 (*Me*CO); 19.9 (*Me*CO). HR-ESI-MS: 447.1712 ([*M* + Na]$^+$, $C_{16}H_{24}N_8NaO_6^+$; calc. 447.1717).

*2,4-Bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-(β-*D*-ribofuranosyl)pyrimidine* (**3**). Commercial 5-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (2.05 mmol, 0.500 g) was dissolved in a 1:2 (*v/v*) mixture (30 ml) of freshly dist. Ac_2O and dry pyridine. The mixture was stirred at r.t. for 24 h, and the volatiles were then removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 ml), washed with sat. $NaHCO_3$ (50 ml), and the org. phase was dried (Na_2SO_4). All volatiles were removed under reduced pressure. The solid residue was dried *in vacuo* to yield 5-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrimidine-2,4-(1*H*,3*H*)-dione (1.7 mmol, 0.630 g, 83%) as white crystals. The product was converted without further purification to *5-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,4-di(1*H*-1,2,4-triazol-1-yl)pyrimidine* (**7**) as described by *Dyatkina et al.* [34]. The yield after CC (SiO_2; CH_2Cl_2 containing 8% MeOH) was 75% (1.27 mmol, 0.600 g). The identity of the product was verified by ESI-MS: 495.16 ([*M* + Na]$^+$, $C_{19}H_{20}N_8NaO_7^+$; calc. 495.14).

Compound **7** (0.42 mmol, 0.200 g) was dissolved in $NH_2NH_2 \cdot H_2O$ (2 ml). The mixture was stirred at r.t. for 48 h, and the remaining NH_2NH_2 were then removed under reduced pressure. Dry pentane-2,4-dione (51 mmol, 5 ml) and CF_3COOH (0.029 μmol, 5 μl) were added, and the mixture was stirred for next 24 h at r.t. The volatiles were removed under reduced pressure, and the residue was purified by HPLC (*LiCroCART® 250–10* column; aq. $AcONH_4$/MeCN 74:26 (*v/v*)). The buffer constituents were removed under reduced pressure to give **3** (0.022 mmol, 0.086 g, 51%). ^{1}H-NMR (400 MHz, D_2O): 8.99 (*s*, H–C(6)); 6.03 (*s*, H–C(4″)); 5.95 (*s*, H–C(4″)); 5.03 (*d*, *J* = 5.3, H–C(1′)); 4.00–4.06 (*m*, H–C(2′,3′)); 3.70 (*dd*, *J* = 4.5, 4.5, H–C(4′)); 3.80 (*dd*, *J* = 4.5, 12.4, H–C(5′)); 3.70 (*dd*, *J* = 4.5, 12.4, H–C(5″)); 2.26 (*s*, Me); 2.18 (*s*, Me); 2.09 (*s*, 2 Me). ^{13}C-NMR (100 MHz, D_2O): 160.6 (C(6)); 156.2 (C(3″)); 155.2 (C(3″)); 152.9 (C(5″)); 152.3 (C(4)); 143.8 (C(5″)); 143.4 (C(2)); 124.1 (C(5)); 110.5 (C(4″)); 108.9 (C(4″)); 83.8 (C(3′)); 77.6 (C(1′)); 76.8 (C(4′)); 71.0 (C(2′)); 61.4 (C(5′)); 13.9 (Me); 12.5 (Me); 12.3 (Me); 11.5 (Me). HR-ESI-MS: 423.1735 ([*M* + Na]$^+$, $C_{19}H_{24}N_6NaO_4^+$; calc. 423.1757).

*2,4-Bis(1-methylhydrazinyl)-5-(β-*D*-ribofuranosyl)pyrimidine* (**4**). Compound **7** (0.42 mmol, 0.200 g) was dissolved in $MeNHNH_2$ (2 ml), and the mixture was stirred at r.t. for 7 d. The volatiles were removed under reduced pressure, and the residue was purified by HPLC (*LiCroCART® 250–10*; aq. $AcONH_4$/MeCN 96:4 (*v/v*)). The buffer constituents were removed under reduced pressure to afford **4** (0.23 mmol, 0.069 g, 55%). ^{1}H-NMR (400 MHz, D_2O): 7.90 (*s*, H–C(6)); 5.59 (br. *s*, H–C(1′)); 4.15 (br. *d*, *J* = 3.6, H–C(2′)); 3.90–3.92 (*m*, H–C(3′,4′)); 3.88 (*dd*, *J* = 1.7, 12.5, H–C(5′)); 3.70 (*dd*, *J* = 3.8, 12.5, H–C(5″)); 3.28 (*s*, MeN); 3.25 (*s*, MeN). ^{13}C-NMR (100 MHz, D_2O): 161.2 (C(4)); 153.3 (C(2)); 139.2 (C(6)); 108.6 (C(5)); 81.7 (C(1′)); 81.0 (C(3′)); 75.8 (C(2′)); 68.9 (C(4′)); 60.5 (C(5′)); 42.2 (MeN); 38.6 (MeN). HR-ESI-MS: 301.1609 ([*M* + H]$^+$, $C_{11}H_{21}N_6O_4^+$; calc. 301.1624).

Chloro[pyridine-2,6-dicarboxamidato(2–)-κ^{3}N^1,N^2,N^6]palladate(1–); **6**). Commercial dimethyl pyridine-2,6-dicarboxylate (3 mmol, 0.585 g) was subjected to ammonolysis (10 ml of 23% aq. NH_3, 40°, 1 h). The volatiles were removed under reduced pressure. The solid white residue was washed thoroughly with H_2O and dried *in vacuo* to yield *pyridine-2,6-dicarboxamide* (**5**) as white powder in 96% yield (0.475 g). The identity of **5** was checked by ESI-MS: 166.06 ([*M* + H]$^+$, $C_7H_8N_3O_2^+$; calc. 166.06).

Compound **5** (1.21 mmol, 200 mg) was treated with an equal amount of K_2PdCl_4 (1.24 mmol, 400 mg) in aq. soln. The mixture was stirred overnight, the yellow precipitate was separated by centrifugation, washed several times with H_2O, and dried *in vacuo* to give **6** (350 mg, 95%). ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.30 (*t*, *J* = 7.8, H–C(4)); 7.75 (*d*, *J* = 7.8, H–C(3,5)); 6.37 (*s*, 2 CONH). ^{13}C-NMR (100 MHz, (D_6)DMSO): 172.1 (2 C=O); 151.2 (C(2,6)); 143.5 (C(4)); 127.7 (C(3,5)). HR-ESI-MS: 303.9085 (*M*$^-$, $C_7H_5ClN_3O_2Pd^-$; calc. 303.9105).

Molecular Modeling. Energy minimizations were performed by MOPAC2007 using the PM6 Hamiltonian, closed-shell wave function, the EF optimizer, and no solvent [38].

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