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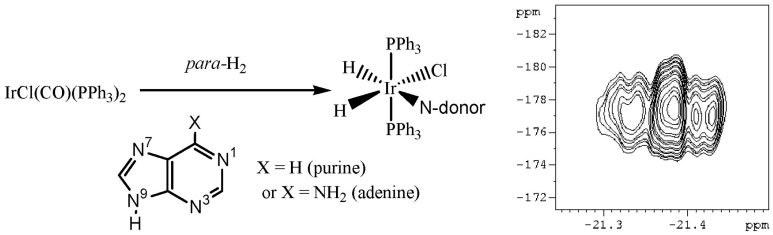


natural abundance $^{1}H$-$^{15}N$ HMQC of N1-purine isomer

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# Detection of Picomole Amounts of Biological Substrates by *para*-Hydrogen-Enhanced NMR Methods in Conjunction with a Suitable Receptor Complex

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Since the discovery of the anti-cancer drug *cis*-platin,[1] and the subsequent accumulation of evidence regarding its mode of action,[2] the reaction chemistry of transition metals toward biological ligands such as DNA nucleotides has been the focus of intense study.[3] Of particular interest is the mode of binding that these ligands exhibit; while DNA itself offers a multiplicity of N- and O-donor sites, it is generally accepted that the remarkable efficacy of *cis*-platin arises from its ability to form intrastrand cross-links, by binding covalently to the $N^7$ sites in guanine.[4] Previous studies have shown the *para*-hydrogen enhancement phenomenon (PHIP) to be a valuable tool in the study of species formed via reactions with hydrogen.[5] Here, we use PHIP to detect picomole amounts of biological substrates by reference to the binding of pyridine, purine, and adenine to $IrCl(H)_2(PPh_3)_2$. This approach provides a sensitivity gain that exceeds that of an NMR cryo-probe by at least 2 orders of magnitude. Optimized detection of DNA base moieties by tip-enhanced Raman methods[6] and HPLC-MS offer similar detection limits.[7]

For this work, NMR tubes were charged with ~0.9 $\mu$mol (0.7 mg) of $IrCl(CO)(PPh_3)_2$, and the biological ligand, 0.12 mmol (10 $\mu$L) of pyridine (py), and made up with $d_8$-toluene. Samples were placed under 3 atm of pure *p*-$H_2$, shaken, and then introduced into the spectrometer at 295 K. The $^1$H NMR spectra obtained under these conditions showed enhanced hydride resonances at $\delta$ −6.86 and −17.58 due to $IrCl(CO)(H)_2(PPh_3)_2$.[8] At 325 K, two new mutually coupled hydride signals due to **1** were observed at $\delta$ −21.03 and −21.82 (Figure 1). Each of these signals appears as a *triplet of antiphase doublets* where the antiphase component arises from coupling between the two former *p*-$H_2$ nuclei. The *triplet* couplings vanish with phosphorus decoupling, and when a $^1$H−$^{31}$P HMQC experiment was recorded, both hydrides' resonances connected to a single $^{31}$P signal at $\delta$ 21.75. These data are consistent with a structure of **1** containing two *trans* phosphine ligands are arranged *cis* to two inequivalent hydride ligands.

Upon repetition of the experiment using $^{13}$CO-labeled $IrCl(CO)(PPh_3)_2$, $^{13}$C-derived couplings to the hydride signals were observed for $IrCl(H)_2(CO)(PPh_3)_2$, but not for **1**, demonstrating the absence of a carbonyl ligand in the new species. Repeating the experiment with 0.03 mmol (2.5 $\mu$L) of $^{15}$N-labeled pyridine gave rise to an extra 17.7 Hz coupling in the hydride resonance at $\delta$ −21.03, consistent with it arising from a proton *trans* to pyridine. Product **1** is therefore $IrCl(H)_2(PPh_3)_2(py)$ (Scheme 1). $^1$H−$^{15}$N HMQC experiments allowed the $^{15}$N resonance to be located at $\delta$ −131 via magnetization transfer from both hydride ligands. Further evidence for the pyridine ligand was obtained by 1D NOE spectroscopy: irradiating the hydride at $\delta$ −21.03 yielded an NOE connection to a $\delta$ 7.86 signal corresponding to an *ortho*-phenyl proton of triphenylphosphine. In contrast, irradiation of the hydride at $\delta$ −21.82 yielded NOE connections to signals at $\delta$ 7.86 and 8.46, thereby demonstrating the spatial proximity of the hydride ligand to both the phosphine ligand and the *ortho* proton of pyridine. Key NMR data for **1** can be found in the Supporting Information. This complex has been partially characterized previously.[9]

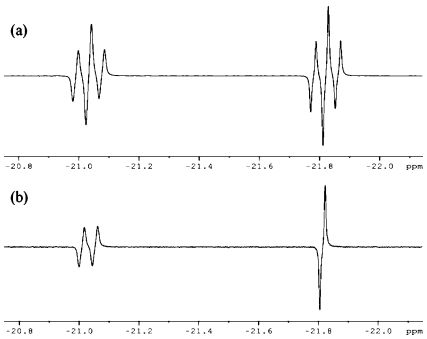
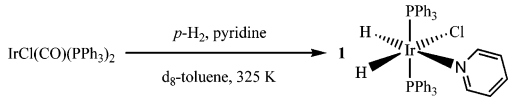



***Figure 1.*** Hydride region of $^1$H NMR spectra obtained from a sample of $IrCl(CO)(PPh_3)_2$ and *p*-$H_2$ at 325 K containing (a) pyridine and (b) $^{15}$N-pyridine (now $^{31}$P decoupled).

***Scheme 1.*** Formation of $IrCl(H)_2(PPh_3)_2$(pyridine) **1**




To learn about the methods sensitivity to detecting pyridine appropriate samples were prepared. After 32 scans at 325 K and 400 MHz (~1 min of acquisition), a signal-to-noise ratio of 11 was obtained for the hydride resonances of **1** when the pyridine concentration was 0.1 $\mu$M, diagnostic of 50 pmol of substrate.

To test the response of the system to a more sterically demanding substrate, pyridine was replaced by benzimidazole. In the associated $^1$H NMR spectra, hydride signals due to **2**, the benzimidazole analogue of **1**, could be seen at $\delta$ −19.22 and −22.19. The $^{15}$N chemical shift of the coordinated nitrogen atom of the five-membered ring of **2** was located on a non-enriched sample at $\delta$ −137.

The $IrCl(CO)(PPh_3)_2$ system was then tested as a sensor for purine. An NMR tube containing *p*-$H_2$, 4.2 $\mu$mol of purine, and $IrCl(CO)(PPh_3)_2$ yielded six new hydride signals at 305 K due to three products, **3a-c**, isomers of $IrCl(H)_2$(purine)$(PPh_3)_2$. The hydride resonances for **3a** ($\delta$ −21.28 and −22.70) are of a much greater magnitude than those for **3b** ($\delta$ −20.86 and −21.82), which are themselves of slightly greater magnitude than those for **3c** ($\delta$ −19.57 and −21.86); assuming that each product is enhanced to the same degree, integration of the resonances indicates that **3a**, **3b**, and **3c** are formed in an approximate ratio of 100:18.6:0.8 at 305 K. Figure 2 illustrates the corresponding spectrum at 325 K. The chemical shifts and coupling parameters of these products closely match those of **1** and **2**, indicating that each of the new species is formed from purine ligating through a nitrogen atom; these assignments were confirmed using a range of $^1$H−$^1$H COSY and $^1$H−$^{31}$P HMQC experiments.

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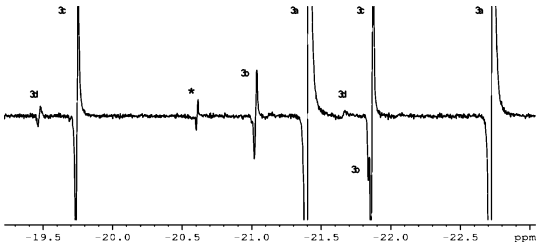
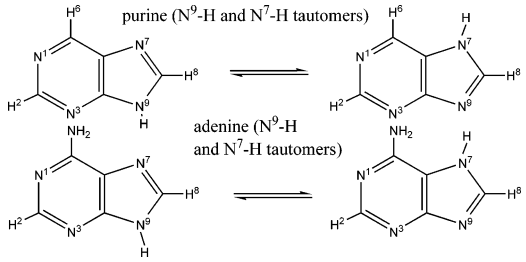



***Figure 2.*** Hydride region of the $^1H\{^{31}P\}$ NMR spectrum of a sample of $IrCl(CO)(PPh_3)_2$ containing 0.5 mg of purine and *p*-$H_2$ at 325 K. The signal marked with ∗ is due to $IrCl(H)_2(PPh_3)_3$.

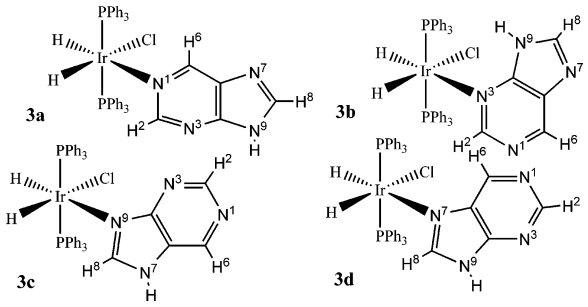
***Scheme 2.*** Tautomers of Purine and Adenine




Scheme 2 shows the principal tautomers of purine with theoretical studies suggesting that the $N^9$-H tautomer is most stable.[10]

The ligand arrangements in products **3a**−**d** (Scheme 3) were partially assigned by comparison of their $^1H$ NMR data with that of **1** and **2** since inspection of the chemical shifts of the *trans*-to-nitrogen hydride signals shows that this parameter is diagnostic of the type of heterocyclic ring in which the ligating nitrogen is situated: hydride signals in the region $\delta$ −19.2 to −19.8 arise from coordination via a five-membered ring, whereas those at $\delta$ −21.0 to −21.5 arise from coordination via a six-membered ring. In the case of **3a**−**d**, it is therefore evident that **3a** and **3b** are species where the purine ligand is bound via the pyrimidine moiety, while **3c** and **3d** contain purine bound via the imidazole moiety. The binding mode of the purine ligand was further determined by NOE spectroscopy where connections for the resonance of the hydride *trans* to chloride provided the crucial information. This was further supported by comparison of the $^1H$ chemical shifts of the coordinated purine with those of free purine. For **3a**, the $\delta$ −22.66 hydride signal shows two NOE interactions to purine ligand protons which resonate at $\delta$ 8.98 and 8.65; in **3a** the purine is bound via $N^1$ because this is the only binding site which has two adjacent protons ($H^6$ and $H^2$). In free purine, these protons usually resonate (in $d_6$-DMSO) at $\delta$ 9.21 and 8.99, respectively; it appears that, upon coordination, the resonances of the protons are shifted *upfield* by approximately 0.3 ppm relative to the free purine. The $N^1$ site of purine is the most basic and least sterically hindered nitrogen donor site in the molecule, which is consistent with the formation of the $N^1$-ligated product **3a** as the most abundant product.[3] The PHIP attained in the case of **3a** proved to be great enough to allow the rapid location of a $^{15}N$ signal at $\delta$ −177 for the coordinated $N^1$ nitrogen with the nucleus *at natural abundance*, which means that signals equating to the detection of femtomole quantities are seen when signal averaging is employed. For **3b**, although the signals are of lower intensity than those of **3a**, the $\delta$ −21.80 hydride signal shows one NOE interaction arising from protons on the purine ligand, at $\delta$ 8.64. This supports the assignment of **3b** as a product arising from $N^3$ coordination of purine, as only the $H^2$ proton is adjacent to the binding site; again, coordination shifts the $H^2$ resonance approximately 0.3 ppm *upfield* from its free ligand value. The relatively small magnitude of the hydride signals of **3b** are consistent with this as the $N^3$ position is the least basic and most

***Scheme 3.*** Structures of the $N^1$-, $N^3$-, $N^9$-, and $N^7$-Coordinated Purine Complexes **3a**, **3b**, **3c**, and **3d**




sterically hindered site. For **3c**, the $\delta$ −21.86 hydride signal exhibits one NOE connection to a ligated purine resonance at $\delta$ 8.45. Since the $H^8$ proton usually resonates at approximately $\delta$ 8.7, it is evident that this signal has also been shifted approximately 0.3 ppm *upfield* upon coordination. However, the assignment of this signal to the $H^8$ proton does not distinguish between the $N^7$-ligated product and the $N^9$-ligated product since both would be expected to show an NOE to $H^8$. In this case, the *absence* of another NOE interaction in the spectrum due to $H^6$ allows **3c** to be assigned as containing an $N^9$-ligated purine ligand. Unfortunately, the hydride signals of **3d** are of insufficient magnitude to conduct NOESY experiments; however, the $\delta$ −19.43 chemical shift of the *trans*-to-nitrogen hydride signal, along with the elimination of the other N-donor sites, allows this species to be identified as the $N^7$-coordinated isomer of $IrCl(H)_2(purine)(PPh_3)_2$.

When the analogous reaction with adenine was examined, the resultant $^1H$ NMR spectrum (325 K) contained dominant PHIP-enhanced hydride signals arising from **4a**, at $\delta$ −21.37 and $\delta$ −22.74, for **4b** at $\delta$ −20.90 and for **4c** at $\delta$ −20.69 and $\delta$ −22.22. These are due to the $N^1$-, $N^3$-, and $N^9$-linkage isomers, respectively. Coordination of the $N^3$ site of adenine as found in **4b** has only previously been reported when the other sites are blocked.[11]

In this report, we establish that *p*-$H_2$-based NMR methods in conjunction with a suitable reporter complex can be used to detect picomole quantities of nucleobases via the detection of diagnostic signals in a normally unoccupied region of the proton NMR spectrum. The sensitivity of the PHIP-enhanced method compares favorably with HPLC-MS for the detection of such species.

**Supporting Information Available:** Synthetic details and key NMR observations. This material is available free of charge via the Internet at http://pubs.acs.org.

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