Application of Gel-Phase ^{195}Pt NMR Spectroscopy in a Novel Solid-Phase Synthesis of a Primary Amine Dichloroplatinum(II) Complex

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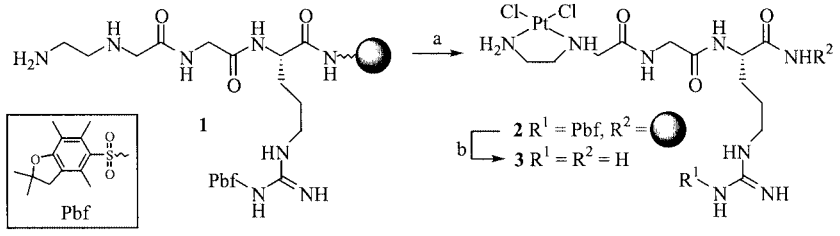
Gel-phase ^{195}Pt NMR spectroscopy of dichloroplatinum peptide complexes attached to a solid support is described. The observed ^{195}Pt chemical shifts of the support-bound platinum complexes are in good agreement with the solution-state ^{195}Pt NMR spectra of the soluble and deprotected analogs. This non-destructive analytical method for on-resin-compound analysis was applied in the optimization of the solid-phase synthesis of the primary amine dichloroplatinum complex **6**, which represents a new class of platinum complexes now available via solid-phase synthetic chemistry.

Introduction

Both combinatorial chemistry and parallel synthesis have embraced solid-phase synthesis (SPS) as one of their fundamental tools. The advantages involved are also likely to pertain to the preparation of libraries of anticancer platinum drugs. Earlier we reported the construction of a dichloroplatinum(II) tripeptide complex (compound **3** in Scheme 1) via a solid-phase approach in nearly quantitative conversion.[1] The rich coordination chemistry of platinum has still not been fully exploited in a solid-phase combinatorial approach. Reactions on a solid support usually require different conditions than in solution, and further explorations in this field would clearly profit from an analytical method that allows the on-resin monitoring of a desired coordination reaction. Standard analytical techniques (MS, HPLC, TLC, NMR) for resin reaction optimization are available once a reaction product is cleaved from the solid support. However, this process is still time consuming, and may alter the desired species or preclude observation of unstable intermediates. This drawback has been widely recognized by solid-phase chemists and, as a result, a plethora of non-destructive analytical methods have been used for on-resin analysis.[2,3] Gel-phase NMR spectroscopy is regarded as one of the more powerful and information rich methods.[4–7] Although on-resin evaluation of metal coordination using NMR spectroscopy, particularly in the field of immobilized catalysts, is of increasing interest,[8–13] only two examples of gel-phase metal NMR spectroscopy have been reported,[12,13] namely for the ^{119}Sn nucleus.

It was envisaged that the diamagnetic ^{195}Pt ($S = 1/2$) nucleus could be applied for monitoring platinum coordination reactions on a solid support using gel-phase NMR spectroscopy. Such an approach would circumvent the need to study the "outer sphere" of an immobilized platinum complex by gel-phase ^{1}H, ^{13}C or ^{31}P NMR spectroscopy, as it is possible to observe the "core" itself.



Scheme 1. Solid-phase synthesis of platinum complex **3**; reagents and conditions: a) K_2PtCl_4 (5 equiv., 0.05 M), DMF/H_2O (9:1, v/v), 24 h in the dark; b) TFA/H_2O (95:5, v/v), 1 h

This paper reports on the feasibility of gel-phase ^{195}Pt NMR spectroscopy, using the immobilized dichloroplatinum peptide complex **2** (Scheme 1). The potential of this technique for assessing the success of an unknown com-

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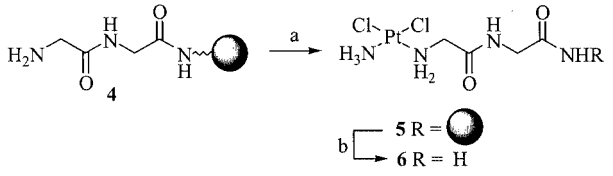
plexation reaction will be illustrated in a novel solid-phase synthesis of the primary amine dichloroplatinum complex **6** (Scheme 2).



Scheme 2. Solid-phase synthesis of platinum complex **6**; reagents and conditions: a) $[PPh_4][PtCl_3(NH_3)]$ (5 equiv., 0.1 M), DMF/H_2O (9:1, v/v) or DMF, 24 h in the dark; b) TFA, 1 h

Results and Discussion

The immobilized peptide dichloroplatinum complex **2** (Scheme 1) was prepared using our previously published method.[1] Gel-phase ^{195}Pt NMR spectroscopy of **2**, preswollen in [D_7]DMF in a 5 mm NMR tube, using standard solution-state parameters, revealed a clear, albeit broad, signal at $\delta = -2336$ ppm (Figure 1A).

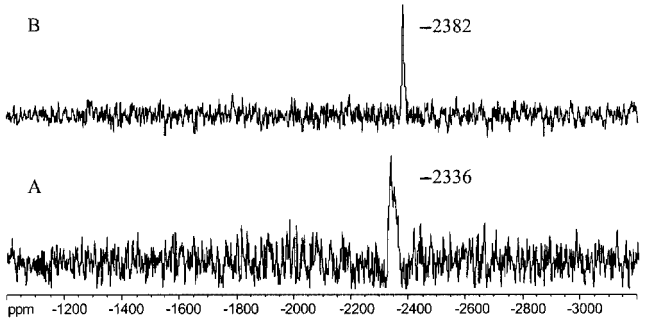


Figure 1. Gel-phase ^{195}Pt NMR spectrum of **2** ([D_7]DMF, 200 mg, 71 μmol) (A), and solution ^{195}Pt NMR spectrum (D_2O) of cleaved and deprotected platinum complex **3** (B)

The gel-phase ^{195}Pt NMR spectrum of **2** not only exhibits a significantly broader signal than the corresponding cleaved and deprotected peptide dichloroplatinum complex **3** dissolved in D_2O (Figure 1B), but is also shifted 46 ppm downfield from the solution-state signal at $\delta = -2382$ ppm. The line width at half-height of **2** is 19.3 ppm, as opposed to a line width at half-height of 6.7 ppm for **3**, indicating that the signal of **2** corresponds to a polymer-bound species. Indeed, when the [D_7]DMF layer was separated from the resin and subjected to ^{195}Pt NMR spectroscopy, no signal appeared. The main factors contributing to line broadening in the gel-phase NMR spectra of polymer-bound species are known to be limited motional freedom, increased correlation time, and magnetic susceptibility variations within the sample.[5,7] The optimal mobility of **2** was attained through the use of DMF, which ensures a high degree of swelling of the polystyrene-based Rink amide resin, as well as solvation of the appended complex with its arginine residue still protected by the apolar Pbf group. Chemical shift studies have indicated that regardless of whether compounds are bound to resins or in solution, the chemical shifts are both virtually identical.[14,15] A conflicting report recently suggested[3] that compounds bound to the solid support exhibit a downfield shift compared to solution-state NMR spectra, due to the high density of aromatic rings within the polymer matrix. However, in the present case the observed, small downfield shift is most likely due to the solvent change, as it has been established that the solvent has a large effect on the chemical shift of platinum (up to a few hundred ppm).[16]

The gel-phase ^{195}Pt NMR technique was subsequently used to optimize the synthesis of a novel type of dichloroplatinum complexes on the solid support. The target molecule **6** (Scheme 2) encompasses a glycine-glycine dipeptide coordinated in a *cis*-configuration to a monoamminedichloroplatinum moiety.

Complex **6** is more cisplatin-like than the dichloroethylenediamineplatinum derivative **3**, as it lacks the ethylene bridge and has a primary amine and a molecule of ammonia as ligands. Gel-phase ^{195}Pt NMR spectroscopy opens the way to investigate whether or not the lack of the ethylenediamine chelating ligand (present in **3**) affects the stability of the primary amine complex on the support. The route of synthesis of target compound **6** (Scheme 2) comprises platination[17,18] of the primary amine of immobilized dipeptide **4** with $[PPh_4][PtCl_3(NH_3)]$, and subsequent release from the solid support with trifluoroacetic acid (TFA). First, dipeptide **4** was reacted with $[PPh_4][PtCl_3(NH_3)]$ (0.1 M, 5 equiv.) in DMF/water (9:1) for one day. The platination conditions were similar to those used for the preparation of **2**, except for the starting platinum salt (K_2PtCl_4 vs. $[PPh_4][PtCl_3(NH_3)]$). After washing the resin with water, DMF, and dichloromethane, a gel-phase ^{195}Pt NMR spectrum was recorded in [D_7]DMF (Figure 2A). The signal at $\delta = -2150$ ppm, with a line width at half-height of 20.9 ppm, corresponds to immobilized **5**, indicating that the solution synthesis method[17,18] is compatible with solid-phase chemistry. However, the signal at $\delta = -1786$ ppm (line width at half-height of 17.8 ppm) was unexpected and is ascribed to hydrolysis of the dichloroplatinum functionality in **5** by the water (10%) present in the reaction mixture.[19] Indeed, subsequent platination in the absence of water afforded a gel-phase ^{195}Pt NMR spectrum devoid of a hydrolysis signal with only the expected $PtCl_2N_2$ chromophore at $\delta = -2147$ ppm (line width at half-height of 15.6 ppm, Figure 2B). This finding indicates that **5** is more susceptible to hydrolysis than the dichloroethylenediamineplatinum derivative **2**, since no hydrolysis signal was observed on the support for the latter after similar reaction conditions. Acidolyis of **5** in TFA and subsequent purification of the product using gel permeation chromatography (HW-40, 0.01 M HCl in water/methanol) gave **6** in 32% yield. The recorded solution-state ^{195}Pt NMR spectrum in D_2O (Figure 2C) shows the $PtCl_2N_2$ chromophore at $\delta = -2200$ ppm, demonstrating roughly the same solvent-induced shift of 53 ppm, as shown for complex **3**.[20] The line width at half-height of **6** (9.6 ppm) is similar to that ob-

served for **3** and considerably smaller than that observed for its immobilized analog **5**.

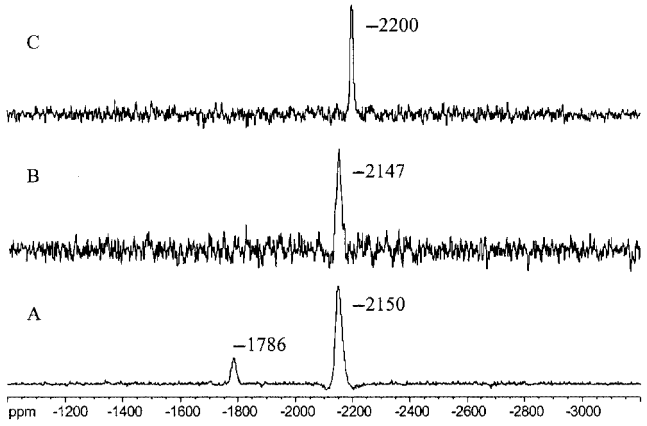


Figure 2. Gel-phase ^{195}Pt NMR spectrum of **5** ([D_7]DMF, 100 mg, 34 μmol) after reaction in DMF/H_2O (9:1) (A), and after reaction in DMF (B); solution-state ^{195}Pt NMR spectrum (D_2O) of cleaved complex **6** (C)

Conclusion

In summary, it has been demonstrated that gel-phase NMR spectroscopy can be applied to the ^{195}Pt nucleus of an immobilized platinum complex. Aided by this technique, the monoamminedichloroplatinum bis-glycine complex **6** has been successfully prepared, representing a new class of platinum complexes now available via solid-phase chemistry. The observed chemical shift of the resin-bound platinum complexes discussed in this paper are in good agreement with the solution-state ^{195}Pt NMR spectra of the soluble and deprotected analogs. As a result of the high receptivity of ^{195}Pt (19.1 times ^{13}C), simple spectra (one signal per nonequivalent Pt) and a wide spectral dispersion (some 15000 ppm, resulting in a high sensitivity to structural perturbations), well-resolved spectra are obtained within hours, without any need for magic-angle spinning. The solvents, polymer backbone and the immobilized ligand are transparent to the technique, eliminating a rigorous drying procedure and suppression techniques. The use of gel-phase ^{195}Pt NMR spectroscopy will be valuable for monitoring a variety of transformations on the resin, aiding the development of novel platinum structures readily available on the solid support, thus facilitating the preparation of new libraries of anticancer platinum complexes.

Experimental Section

General Remarks: All solvents and reagents were of commercial grade and used without purification. Solvents and reagents used in the automated peptide synthesis were of peptide synthesis grade and were purchased from Biosolve. Amino acids and the Rink Amide MBHA resin were purchased from NovaBiochem. K_2PtCl_4 was obtained as a loan from Johnson−Matthey. [PPh_4][$PtCl_3(NH_3)$] was synthesized according to a previously published procedure.[18] Gel permeation chromatography was executed on a HW-40 column (26 mm × 600 mm) at 1.5 mL/min. Mass spectra were recorded on a PE SCIEX API 165 instrument.

NMR Spectroscopy: (Gel-phase) NMR spectra were recorded on a Bruker DPX 300 spectrometer with a 5-mm multi-nucleus probe. The temperature was kept constant at 295 K by a variable temperature unit. ^{1}H NMR spectra were measured using TMS as an external reference at δ = 0 ppm. ^{195}Pt NMR spectra were calibrated with respect to external K_2PtCl_4 at δ = −1614 ppm. Gel-phase ^{195}Pt NMR samples of **2** and **5** were prepared by swelling respectively 200 mg (71 μmol) and 100 mg (34 μmol) of platinated, washed and dried resin in 1 mL of [D_7]DMF in a 5 mm NMR tube. The number of scans used for the gel-phase experiments varied from 89262 to 1243681.

***cis*-Amminedichloro(glycylglycine-NH_2)platinum(II) (6):** Dipeptide **4** was assembled on Rink amide resin with an ABI 433A (Applied Biosystems, division of Perkin−Elmer) peptide synthesizer, employing a FastMoc® peptide synthesis protocol. Immobilized **4** (100.0 mg, 37.9 μmol) was reacted with [PPh_4][$PtCl_3(NH_3)$] (124.6 mg, 189.4 μmol, 0.1 M in DMF) in the dark for one day, washed with water, DMF and dichloromethane and dried in vacuo. Cleavage of the solid support was effected by treatment of **5** with TFA for 1 h, after which the reaction mixture was precipitated with diethyl ether. The resulting solid was washed with diethyl ether, water was added, the mixture was filtered and the resin was washed with water. Subsequently, the combined water fractions were lyophilized, yielding crude **6** as a yellow powder. Purification by gel permeation chromatography (HW-40, 0.01 M HCl in water/methanol, 1:1, v/v) afforded **6** (5.0 mg, 12.0 μmol) in 32% yield, based on **4**. ^{1}H NMR (D_2O): δ = 3.85 (s, 2 H, αGly), 3.83 (s, 2 H, αGly) ppm. ^{195}Pt NMR (D_2O): δ = −2200 ppm. ESI-MS: *m/z* = 449 [M + H + HCl]$^+$.

Acknowledgments

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