Targeting Platinum Compounds: synthesis and biological activity

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Extending solid-phase methods in inorganic synthesis: the first dinuclear platinum complex synthesised via the solid phase*

Abstract - Efficient synthetic methods to assemble dinuclear platinum complexes would speed up the discovery of this class of potentially antitumour active compounds. This chapter describes how dinuclear platinum coordination compounds can be obtained via a straight-forward solid-phase method suitable for parallel synthesis.

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3.1 INTRODUCTION

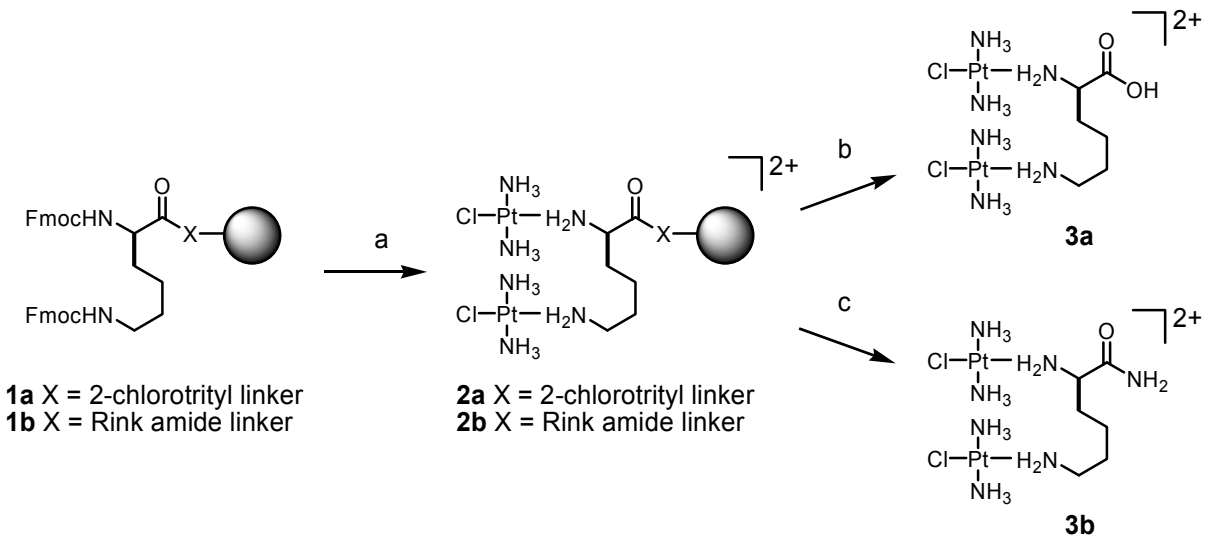
Cisplatin is one of the most widely used anticancer agents. However, as described in Chapter 1, the clinical application of cisplatin is limited by serious side effects, such as nephrotoxicity, neurotoxicity and ototoxicity [1-3]. Furthermore, some tumours are intrinsically resistant to cisplatin, while others acquire resistance during cisplatin treatment. Therefore there is great interest in the development of platinum antitumour drugs that do not display cisplatin resistance [4]. Dinuclear platinum(II) complexes with bridging diamine linkers represent a new class of anticancer agents with high *in vivo* activity, both in cisplatin sensitive and resistant tumours [5,6].

The first example of a solid-phase mediated synthesis of peptide-tethered dichloroplatinum(II) complexes, and the automated synthesis of a family of 36 analogues were recently reported [7,8]. As an extension of these studies the first solid-phase synthesis of dinuclear lysine bridged platinum(II) complexes is described here. Analogous to the dinuclear platinum(II) complexes with bridging diamine linkers, these complexes contain two platinum moieties, separated by a simple diamine bridge. Additionally, the carboxylic acid functionality of lysine provides a handle via which these compounds can be appended to a solid-support and a position at which variations can be made. The presented method may be used to significantly speed up the discovery of new platinum anticancer agents designed to overcome cisplatin resistance. Application of this method in the synthesis of targeting platinum compounds is described Chapter 4.

3.2 SYNTHESIS AND BIOLOGICAL TESTING

A successful solid-phase synthesis of dinuclear platinum(II) complexes with a bridging lysine moiety depends on the availability of a suitable linker, enabling the solid-phase assembly and subsequent cleavage of the target complexes under conditions that do not disrupt the integrity of the platinum moiety. Final acidic cleavage is desirable as it allows the use of standard Fmoc-based SPPS for the assembly of the complexes [9]. In order to investigate the acid-stability of the immobilised target complexes, N-α,ε-di-Fmoc-L-Lysine was condensed with Rink amide MBHA linker to give **1a** and 2-chlorotrityl linker yielding **1b** (Scheme 3.1). The Rink amide linker requires 90-95% TFA for quantitative liberation of the peptide amide, while the 2-chlorotrityl linker will release the free acid in conditions as mild as 1-5% TFA [10].

After treatment of **1a**-**b** with piperidine, the primary amines were platinated with a five-fold excess of *trans*-diamminedichloroplatinum (transplatin), activated by overnight reaction with 0.9 equivalent of $AgNO_3$, leading to the immobilised dinuclear platinum compounds **2a** and **2b**. Gel-phase ^{195}Pt NMR of **2a** in DMF-d_7 shows a single broad signal at -2404 ppm, typical for the [$PtClN_3$] chromophore [11]. Cleavage with 30% acetic acid in DMF followed by precipitation with diethyl ether afforded the desired dinuclear compound **3a**, in 59% yield.

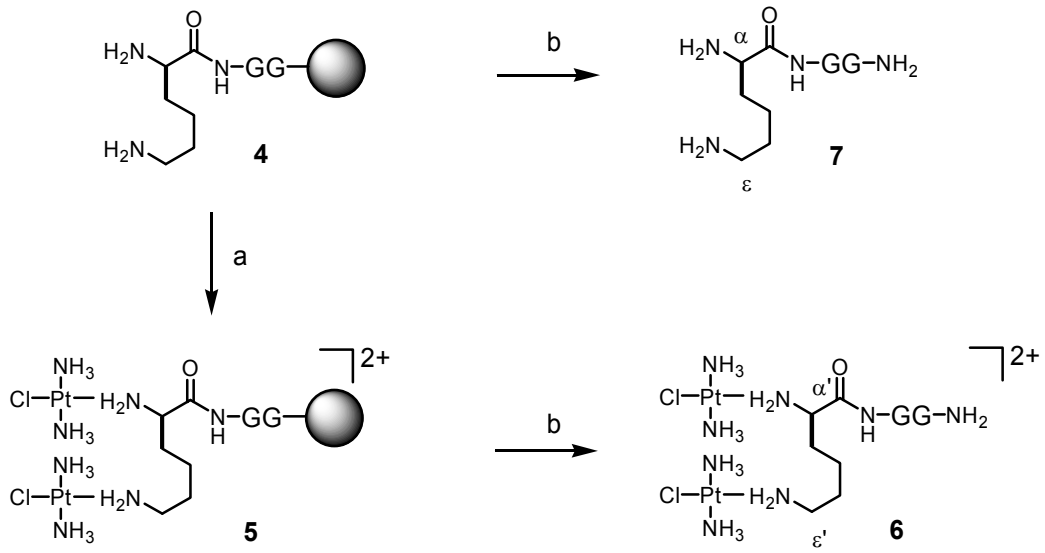


Scheme 3.1: Solid-phase synthesis of platinum complexes **3a** and **3b**. *Reagents and conditions* a) i. piperidine 20% in DMF, ii. *trans*-[$Pt(NH_3)_2Cl(dmf)]^+$ (5 equiv) TEA (7 equiv) in DMF; b) 1 ml 30% AcOH in DMF 2 h rt.; c) 1 ml 95% TFA in H_2O for 1 h rt.

Solution ^{195}Pt NMR measured in D_2O shows a single broad peak with a very similar chemical shift of -2390 ppm. The ^{1}H NMR illustrates the dinuclear nature of the compound **3a** as both

the α- and ε-lysine protons show an upfield shift of 0.3 ppm compared to the free amino acid. When treating **2b** with 95% TFA in water the dinuclear amide platinum complex is cleaved. Precipitation with diethyl ether affords a light yellow compound **3b** that shows two peaks in the ^{195}Pt NMR with chemical shifts of -2397 ppm and -2406 ppm, as expected for the two distinct [$PtClN_3$] moieties present in the complex. Clearly both the 2-chlorotrityl and the Rink amide linkers are suitable for the solid-phase synthesis of dinuclear *trans*-platinum complexes.

To test the suitability of the presented method for the synthesis of more complex molecules a dinuclear platinum moiety tethered to a dipeptide was synthesised. For this purpose the Rink Amide MBHA resin was selected as the solid-phase carrier. Using a standard Fmoc protocol the resin bound tripeptide **4** was formed (Scheme 3.2). Platination of the lysine was achieved with a 5 fold excess of activated transplatin to give the immobilised complex **5**. Complex **5** shows a broad gel-phase ^{195}Pt NMR peak at -2399 ppm (Figure 3.1). Cleavage from the resin with 95% TFA in water gave the desired dinuclear platinum complex **6**.



Scheme 3.2: Solid-phase synthesis of platinum complex **6** and ligand **7**. *Reagents and conditions*
a) *trans*-[$Pt(NH_3)_2Cl(dmf)]^+$ (5 equiv) TEA (7 equiv) in DMF; b) 1 ml 95% TFA in H_2O for 1 h rt.

The crude reaction product was purified on Sephadex G-10 (Pharmacia) and lyophilised to yield a light-yellow solid in 66% yield. The solution ^{195}Pt NMR of compound **6** shows two partly overlapping peaks in close proximity, at -2397 and -2415 ppm (Figure 3.1). Clearly signal broadening due to limited motional freedom of the immobilised compound **5** causes the two signals to overlap completely. In the solution spectrum of **6** on the other hand, the signals are sharper and distinction between the two similar platinum moieties can be made. As for

compound **3a** the ^{1}H NMR of **6** reveals a downfield shift of the α- and ε- protons with respect to the ligand **7** from 3.98 ppm and 3.01 ppm to 3.67 ppm and 2.69 ppm, respectively. During a pH titration followed by ^{1}H NMR, complex **6** shows no pH dependence of the α-proton and the ε- protons unlike the pH titration of the free ligand **7** (Figure 3.2). This indicates that the terminal amines can no longer be protonated, and therefore must be coordinated to the platinum moieties.

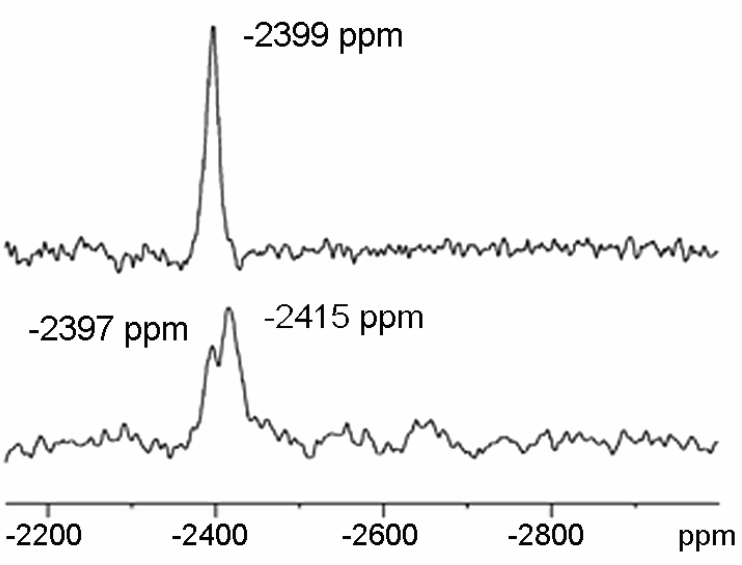


Figure 3.1: Gel-phase (above) and solution state (below) ^{195}Pt NMR of **5** and **6**, respectively.

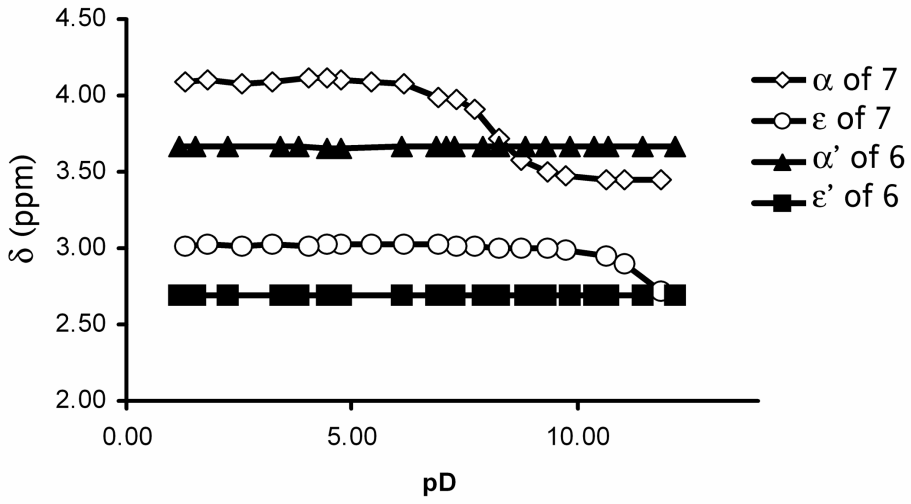


Figure 3.2: pH titration of free ligand **7** and complex **6** showing the pH dependence of the chemical shift of the α-and ε-protons.

The cytotoxic behaviour of **6** was studied in A2780 human ovarian cancer cell lines sensitive and resistant to cisplatin. With an IC_{50} value around 80 μM, the compound shows a 25 fold decrease in activity with respect to cisplatin and $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-}H_2N(CH_2)_5NH_2)]^{2+}$, the dinuclear compound without appended peptide, in the sensitive cell line. In the resistant cell line no significant cytotoxic effect was observed at drug concentrations up to 100 μmol so the complex clearly does not overcome cisplatin resistance in this cell line. Apparently the appended di-glycine renders the compound much less cytotoxic, possibly related to reduced cellular uptake or changed DNA-binding properties.

3.3 CONCLUSION

The presented results show that not only cisplatin analogues, but also dinuclear platinum complexes can be synthesised using SPPS, when combined with synthetic techniques used in platinum chemistry. The resulting complexes are formed easily in high yields, and biological testing shows their potential as anticancer agents. Through the use of solid-phase techniques the speed with which new dinuclear platinum anticancer drug candidates can be synthesised is dramatically increased. By synthesising platinum-peptide conjugates using biologically active peptides, such as peptides that show selective uptake into cancer tissue, or peptides that can shuttle drugs towards nuclear DNA, targeted platinum drugs can be obtained as described in Chapter 4 of this thesis.

3.4 EXPERIMENTAL SECTION

3.4.1 General

Chemicals and solvents were purchased from Acros, Nova-Biochem and Biosolve and used as received unless otherwise stated. *Trans*-$[Pt(NH_3)_2Cl_2]$ was obtained using literature procedures, from K_2PtCl_4, provided by Johnson and Matthey on a generous loan scheme. All NMR measurements were performed on a 300 MHz Bruker DPX300 spectrometer with a 5 mm multi-nucleus probe. Temperature was kept constant at 298 K using a variable temperature unit. ^{1}H and ^{195}Pt chemical shifts were referenced to TSP and Na_2PtCl_4 (δ = 0 ppm), respectively. The water signal for spectra measured in D_2O was minimized using a WATERGATE pulse sequence. MS spectra were taken on a ThermoFinnegan AQA ESI-MS.

3.4.2 Synthesis of the complexes

1a: To preswollen 2-chlorotrityl resin (0.05 mmol) was added , N-α,ε-di-Fmoc-L-Lysine OH (0.1 mmol, 2 equiv) and DIPEA (0.2 mmol) in DCM. After 1.5 h the resin was washed (DCM/MeOH/DIPEA 17:2:1; DCM, DMF, DCM) to yield **1a**.

2a: *trans*-$[Pt(NH_3)_2Cl_2]$ (0.25 mmol, 5 equiv) was activated by treatment with $AgNO_3$ (0.24 mmol, 4.8 equiv) in DMF (1.5 ml) overnight in the dark. AgCl was removed by filtration. After treatment of the preswollen resin **1a** with 20% piperidine in DMF (2 x 1 ml for 10 min) and washing (3 x 5 min DMF) the transplatin solution was added. TEA was added (0.35 mmol, 7 equiv) and the mixture was shaken overnight in the dark to yield **2a**. ^{195}Pt NMR (DMF-d_7): δ (ppm): -2404.

3a: Complex **2a** was treated with 30% AcOH in DMF 2 h at rt. The product was precipitated with diethyl ether. The resulting solid was filtered, washed with diethyl ether and redissolved in water. Filtration and lyophilisation gave the desired product (20 mg, 0.03 mmol) in 59% yield. ^{1}H NMR (D_2O) δ (ppm): 1.45 (t, J = 7.6 Hz, 2H; γK), 1.72 (m, 4H; βK, δK), 2.68 (t, J = 7.2 Hz, 2H; εK), 3.42 (m, 1H, αK). ^{195}Pt NMR (D_2O): δ -2390. ESI-MS: *m/z*: 674.0 $[M+H]^+$, 337.0 $[M+H]^{2+}$.

1b: Fmoc protected Rink Amide MBHA resin was treated with 20% piperidine in DMF (2 x 20 min) after which the resin was washed (DMF, DCM). N-α,ε-di-Fmoc-L-Lysine OH (0.2 mmol, 4 equiv), BOP (0.2 mmol) and DIPEA (0.4 mmol) in NMP (1 ml) was added and the reaction was shaken for 1 h. The resin was washed (NMP, DCM, NMP) to yield **1b**.

2b: *trans*-$[Pt(NH_3)_2Cl_2]$ (0.25 mmol, 5 equiv) was activated by treatment with $AgNO_3$ (0.24 mmol, 4.8 equiv) in DMF (1.5 mL) overnight in the dark. AgCl was removed by filtration. After treatment of the preswollen resin **1b** with 20% piperidine in DMF (2 x 1 ml for 10 min) and washing (3 x 5 min DMF) the transplatin solution was added. TEA was added (0.35 mmol, 7 equiv) and the mixture was shaken overnight in the dark to yield **2b**.

3b: Cleavage was effected by treatment of **2b** with 95% TFA in water. The desired product was obtained as a yellow powder (15 mg, 0.02 mmol) in 40% yield. ^{1}H NMR (D_2O) δ (ppm): 1.37 (m, 2H; γK), 1.72 (m, 4H; βK, δK), 2.61 (m, 2H; εK), 3.44 (m, 1H, αK). ^{195}Pt NMR (D_2O): δ (ppm) -2397, -2406.

4: Fmoc-protected Rink Amide MBHA resin was treated with 20% piperidine in DMF (2 x 20 min) after which the resin was washed (DMF, DCM). (0.2 mmol, 4 equiv), BOP (0.2 mmol) and DIPEA (0.4 mmol) in NMP (1 ml) was added and the reaction was shaken for 1 h. The resin was washed (NMP, DCM, NMP). Removal of the Fmoc group was accomplished by treatment with 20% piperidine in DMF (2 x 20 min) after which the resin was washed (DMF, DCM). The subsequent N-α Fmoc-L-Glysine OH and N-α,ε-di-Fmoc-L-Lysine OH were coupled under analogous conditions in a stepwise procedure to yield **4**.

5: *trans*-$[Pt(NH_3)_2Cl_2]$ (0.25 mmol, 5 equiv) was activated by treatment with $AgNO_3$ (0.24 mmol, 4.8 equiv) in DMF (1.5 mL) overnight in the dark. AgCl was removed by filtration.

After treatment of the preswollen resin **4** with 20% piperidine in DMF (2 x 1 mL for 10 min) and washing (3 x 5 min DMF) the transplatin solution was added. TEA was added (0.35 mmol, 7 equiv) and the mixture was shaken overnight in the dark to yield **5**. ^{195}Pt NMR (DMF-d_7): δ (ppm) -2399 ppm.

6: Treating **5** with 95% TFA and precipitation with diethyl ether the crude product was obtained (54 mg). This was dissolved in water and poured on a Sephadex G-10 (Pharmacia) column (3 x 11 cm, solvent LiCl (1 M), flow rate 0.85 mL/min, detection UV (245 nm)) to be purified and lyophilized. The resulting powder was used for analysis and testing (26 mg, 0.033 mmol, 66% yield). ^{1}H NMR (D_2O) δ (ppm): 1.42 (m, 2H; γK), 1.68-1.82 (broad m, 4H; δK, βK), 2.69 (t, J = 6.9 Hz, 2H; εK), 3.66 (m, 1H, αK), 3.93, 4.11 (ds, 4H; αG). ^{195}Pt NMR (D_2O): δ (ppm) -2397, -2415. ESI-MS: *m/z*: 788.5 $[M+H]^+$, 394.2 $[M+H]^{2+}$.

7: Treatment of **4** with 95% TFA in water and subsequent precipitation in diethyl ether gave **7** as a white powder. ^{1}H NMR (D_2O) δ (ppm): 1.48 (m, 2H; γK), 1.72 (m, 2H; δK), 1.88 (m, 2H; βK), 3.01 (t, J = 7.5 Hz, 2H; εK), 3.94, 4.05 (ds, 4H; αG), 3.98 (m, 1H, αK).

3.4.3 pH titration

The pH titration was performed in a D_2O solution by adjustment of pD using DCl and NaOD. ^{1}H chemical shifts were referenced to TMA (3.18 ppm). pD values were measured at 298 K using a PHM 80 pH meter (Radiometer) before and after each ^{1}H NMR measurement. The pH values were not corrected for the H/D isotope effect.

3.4.4 Growth inhibition assays in A2780 and A2780R

A2780 and A2780R human ovarian cell lines were a gift from Dr. J.M. Perez (Universidad Autónoma de Madrid, Spain). Growth inhibition by the complex **6**, $[\{trans\text{-}PtCl_2(NH_3)_2\}(\mu\text{-}H_2N(CH_2)_5NH_2)]^{2+}$ and cisplatin was determined by MTT-based assay. Cells were grown as monolayers in Dulbecco's modified Eagle's Medium supplemented with 10% fetal calf serum (Gibco, Paisley, Scotland), penicillin (100 units/ml: Dufecha, Netherlands) and streptomycin (100 μg/ml: Dufecha, Netherlands).

Cells were pre-cultured for 48 h at 37 ^{o}C in a 7% CO_2 containing incubator in 96 multi-well plates and subsequently treated with 100 μl of compound at six different concentrations from 50 to 0 μmol in quadruplicate. After 72 h, MTT in PBS (100 μl at 2.5 mg/ml) was added and the cells were incubated for 2 h. The solution was carefully removed and the remaining crystals dissolved in 100 μl of DMSO after which the absorbance at 590 nm of each well was determined using a plate reader. The growth inhibition was determined relative to untreated controls. The experiments were performed in triplicate.

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