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

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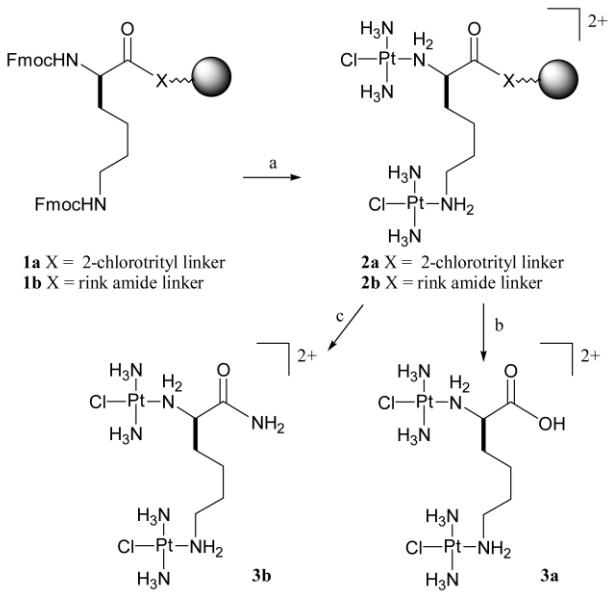
A method for obtaining potentially anti-tumour active dinuclear platinum coordination compounds *via* solid-phase inorganic synthesis is described for the first time.

Cisplatin is one of the most widely used anticancer agents. The clinical application of cisplatin, however, 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 anti-tumour 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]

We recently reported the first example of a solid-phase mediated synthesis of peptide-tethered dichloroplatinum(II) complexes,[7] and the automated synthesis of a family of 36 analogues.[8] As an extension of these studies we here describe the first solid-phase synthesis of dinuclear lysine bridged platinum(II) complexes. The presented method may be used to significantly speed up the discovery of new platinum anticancer agents designed to overcome cisplatin resistance.

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 1). Rink-amide requires 90–95% TFA for quantitative liberation of the peptide amide, while 2-chlorotrityl will release the free acid in conditions as mild as 1–5% TFA.[10]

After treatment of **1a**,**b** with piperidine, the primary amines where platinated with a five-fold excess of *trans*-diamminedichloroplatinum (transplatin), activated by overnight reaction with 0.9 equiv. of $AgNO_3$, leading to the immobilised dinuclear platinum compounds **2a** and **2b**. Gel-phase ^{195}Pt NMR of **2a** in d^7-DMF 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 ether afforded the desired dinuclear compound **3a** in 59% yield. 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 ether afforded 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 transplatinum complexes.‡



1a X = 2-chlorotrityl linker
1b X = rink amide linker
2a X = 2-chlorotrityl linker
2b X = rink amide linker

Scheme 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.

To test the suitability of the presented method for the synthesis of more complex molecules we synthesised a dinuclear platinum moiety tethered to a dipeptide. For this purpose we selected the Rink Amide MBHA resin as the solid-phase carrier. Using a standard Fmoc protocol the resin bound tripeptide **4** was formed (Scheme 2). Platination of the lysine was achieved with a 5 fold excess of activated transplatin to give the immobilised compound **5**. Compound **5** shows a broad gel-phase ^{195}Pt NMR peak at −2399 ppm (Fig 1). Cleavage from the resin with 95% TFA in water gave the desired dinuclear platinum complex **6**.

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 (Fig 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** (Fig. 2). This indicates that the terminal amines can no

† Electronic supplementary information (ESI) available: Definitions for abbreviations used, and synthesis of **1a**, **1b**, **4** and **7**. See http://www.rsc.org/suppdata/cc/b2/b212388f/

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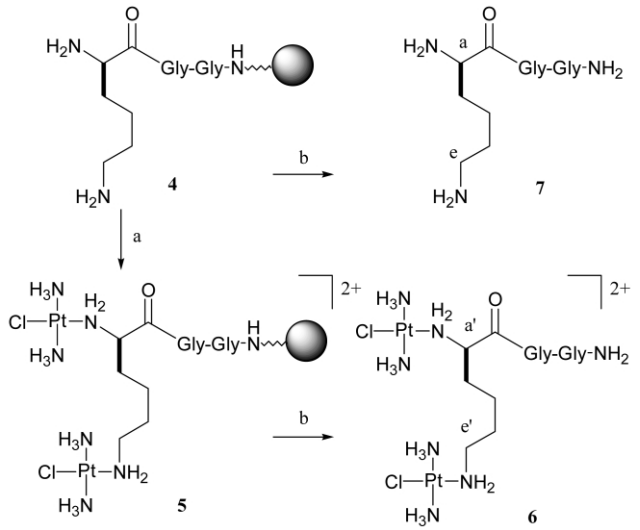

Scheme 2 Solid-phase synthesis of platinum complex **6** and ligand **7**. *Reagents and conditions* a) *trans*-$[Pt(NH_3)_2Cl(dmf)]^+$ (5 equiv.) TEA (7 equiv.) in DMF; b) 1 mL 95% TFA in H_2O for 1 h rt.

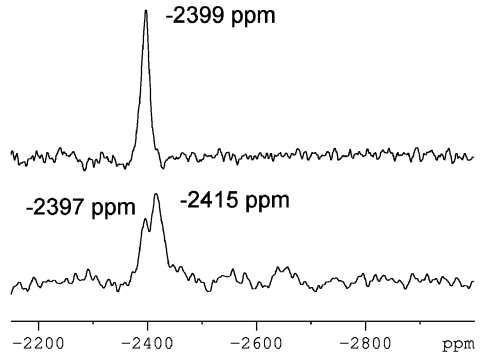

Fig. 1 Gel-phase (above) and solution state (below) ^{195}Pt NMR of **5** and **6** respectively.

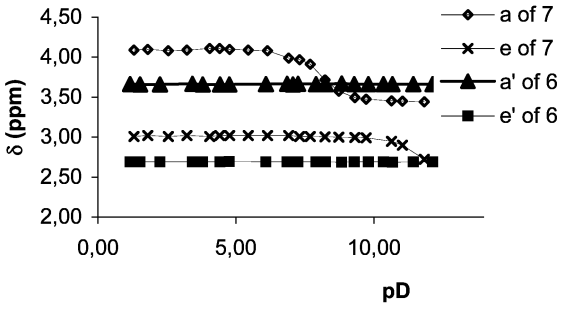

Fig. 2 pH titration of free ligand **7** and complex **6** showing the pH dependence of the chemical shift of the α- and ε-protons.

longer be protonated, and therefore must be coordinated to the platinum moieties.

The cytotoxic behaviour of **6** was studied in A2780 human ovarian cancer cell lines sensitive and resistant to cisplatin. The compound showed a 60 fold decrease in activity with respect to cisplatin and $[\{trans\text{-}PtCl_2(NH_3)_2\}(\mu\text{-}H_2N(CH_2)_5NH_2)]^{2+}$, 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. Clearly the compound is much less cytotoxic than cisplatin and does not overcome cisplatin resistance in this cell line.

The presented results show that not only cisplatin analogues, but also dinuclear platinum complexes can be synthesised using SPPS, 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. The presented technique is currently being used to synthesise small focused libraries of platinum drugs tethered to different targeting devices.

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Notes and references

‡ 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. ^1H 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.

Synthesis of 3a: *trans*-$Pt(NH_3)Cl_2$ (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 × 1 mL for 10 min) and washing (3 × 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 (d^7-DMF): δ −2404 ppm.

Cleavage from the resin was effected by treatment with 30% AcOH in DMF 2 h at rt. The product was precipitated with diethyl ether. The resulting solid was washed with diethyl ether and redissolved in water. Filtration and lyophilisation gave the desired product (20 mg, 0.03 mmol) in 59% yield. Non-exchangeable protons assigned by ^1H NMR; ESI-MS: *m/z*: 674.0 $[M]^+$, 337.0 $[M]^{2+}$; ^{195}Pt NMR (D_2O): δ −2390 ppm.

Synthesis of 3b: as for **3a** where 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. Non-exchangeable protons assigned by ^1H NMR; ^{195}Pt NMR (D_2O): δ −2397 ppm, −2406 ppm).

Synthesis of 6: Synthesis of **4** is described in the ESI†. Platination and cleavage was accomplished as for **3b** to give the crude product (54 mg). This was dissolved in water and poured on a Sephadex G-10 (Pharmacia) column (3 × 11 cm, solvent LiCl (1 M), flow rate 0.85 mL min^{-1}, detection UV (245 nm)) to be purified and lyophilised. The resulting powder was used for analysis and testing (26 mg, 0.033 mmol, 66% yield). **5**: ^{195}Pt NMR (d^7-DMF): δ −2399 ppm. **6**: Non-exchangeable protons assigned by ^1H NMR; ESI-MS: *m/z*: 788.5 $[M]^+$, 394.2 $[M]^{2+}$; ^{195}Pt NMR (D_2O): δ −2397 ppm, −2415 ppm.

pH titration: The pH titration was performed in a D_2O solution by adjustment of pD using DCl and NaOD. ^1H 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 ^1H NMR measurement. The pH values were not corrected for the H/D isotope effect.

Growth inhibition assays in A2780 and A2780cisR: A2780 and A2780cisR human ovarian cell lines were a gift from Dr J. M. Perez (Universidad Autonoma 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 using an MTT-based assay using a previously published method.[12]

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