

Synthesis of DNA-Sequence-Selective Hairpin Polyamide Platinum Complexes

Robin I. Taleb,^[a] David Jaramillo,^[a, b] Nial J. Wheate,^[a] and Janice R. Aldrich-Wright*^[a]

Abstract: Two DNA-sequence-selective hairpin polyamide platinum(II) complexes, containing pyrrole and imidazole heterocyclic rings, have been synthesised by different methods. A six-ring complex, selective for (A/T)GGG-(A/T) DNA sequences, was made by using solid-phase synthesis, whilst an eight-ring complex, selective for (A/T)CCTG(A/T) DNA sequences, was

made by utilising standard wet chemistry. Solid-phase synthesis resulted in a significantly higher yield, required less purification and is more efficient than the wet synthesis; as such, it is the pre-

ferred method for further work. The metal complexes were characterised by ¹H and ¹⁹⁵Pt NMR spectroscopy and ESI mass spectrometry. The two compounds provide a foundation for the synthesis of more complex molecules containing multiple hairpins and/or platinum groups.

Keywords: antitumor agents • DNA recognition • platinum • polyamides • synthesis design

Introduction

The platinum complexes, cisplatin, carboplatin and oxaliplatin, currently used in the clinic for the treatment of a variety of human cancers, are limited by their large-dose-limiting side effects and by acquired or intrinsic resistance.^[1] In an effort to overcome these problems, new drugs are being developed that exhibit different reactivity and/or DNA binding. Multinuclear platinum complexes have shown utility in overcoming resistance,^[2,3] but while encapsulation within protective molecules can reduce their reactivity and degradation,^[4,5] these complexes have not shown significant efficacy in recent phase II clinical trials.^[6,7] The design of mononuclear or multinuclear platinum drugs that are capable of specifically targeting cancerous cells, by binding to mutant genes or extended telomere regions, could simultaneously reduce the side effects and increase efficacy.^[8]

Over the last ten years, polyamides have been synthesised that are capable of targeting specific DNA sequences. These compounds, containing imidazole (Im), pyrrole (Py) and hydroxypyrrole (Hp) heterocyclic rings, along with β -alanine and amino butyric acid (γ -turn linker) residues, can be constructed in different combinations and therefore used to target specific DNA regions.^[9] Each pair of heterocyclic rings recognises a specific base pair in DNA: Im/Py and Py/Im pairings distinguish between G:C and C:G base pairs, respectively, while Hp/Py and Py/Hp recognise T:A and A:T base pairs. The combination of Py/Py is selective for both A:T and T:A base pairs.^[9]

Covalent linking of two antiparallel polyamides results in molecules with increased DNA affinity and sequence specificity. Amino butyric acid connects the carboxylic terminus of one polyamide to the amino terminus of another. Polyamides with γ turns display ≈ 100 -fold increased DNA affinity compared to that of unlinked homodimers. In fact, eight-ring hairpins show affinity and sequence specificity similar to DNA-binding proteins (dissociation constant $K_d < 1$ nM).^[10] The γ -turn linker locks the register of the ring pairings, thereby preventing the possibility of slipped-dimer formation, that is, the polyamide binding to DNA in either a 2:1 or 1:1 ligand-DNA binding mode.^[11] Hairpin compounds retain orientation preferences and align N \rightarrow C with respect to the 5' \rightarrow 3' direction of the adjacent DNA strand.^[12]

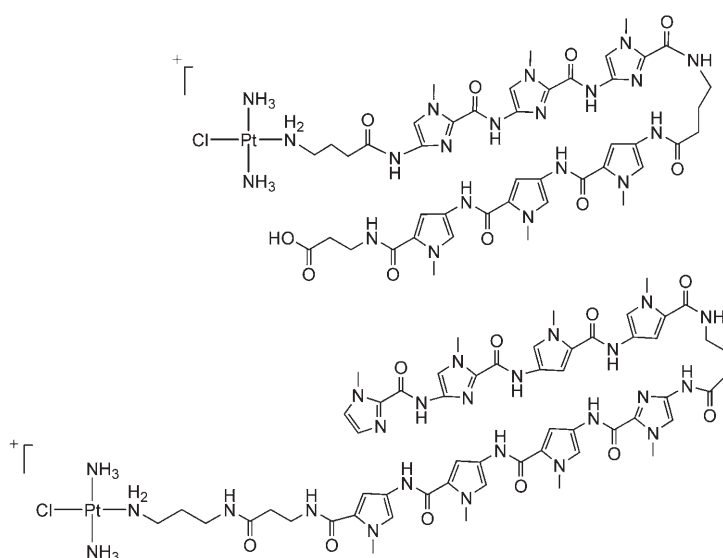
We have previously examined the potential of single-stranded polyamides linked to a platinum group capable of coordinate covalent bond formation with DNA.^[8] Higher

[a] R. I. Taleb, Dr. D. Jaramillo, Dr. N. J. Wheate, Assoc. Prof. J. R. Aldrich-Wright
School of Biomedical and Health Sciences
University of Western Sydney
Locked Bag 1797, Penrith South DC, NSW (Australia)
Fax: (+61)2-4620-3025
E-mail: j.aldrich-wright@uws.edu.au

[b] Dr. D. Jaramillo
Current address:
Department of Chemistry and Biochemistry
University of California, San Diego
La Jolla, CA 92093 (USA)

DNA affinity and the ability to prevent DNA transcription may be imparted by instead using a hairpin polyamide linked to a platinum moiety.

The wide availability of knowledge on polyamide synthesis means that the organic components of these compounds can be made by different methods, either solid-phase or standard wet chemistry synthesis. In this paper we report the preparation of two hairpin polyamide platinum complexes; a six-ring polyamide platinum complex, **HLSP-6**, was made by using solid-phase synthesis and an eight-ring polyamide platinum complex, **HLWC-8**, was made by using standard wet chemistry (Scheme 1). We report their characterisation by ^1H and ^{195}Pt NMR spectroscopy and high-resolution mass spectrometry, along with the disadvantages and merits of the alternative synthetic methods.



Scheme 1. Two hairpin polyamide platinum complexes synthesised in this study. **HLSP-6** (top) was made by solid-phase synthesis and **HLWC-8** (bottom) was made by standard wet chemistry.

Results

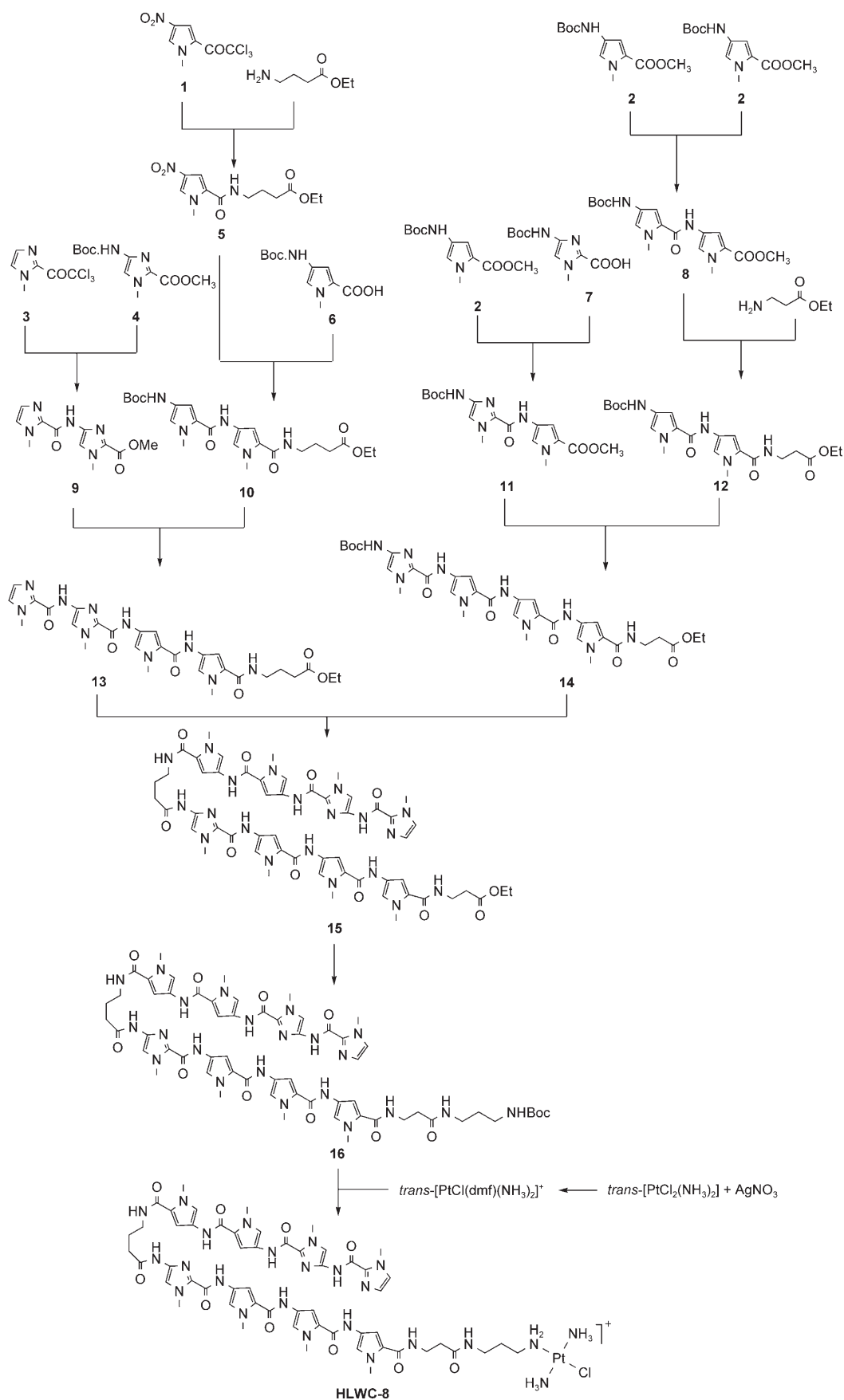
Wet synthesis of HLWC-8: Synthesis of the polyamide framework was achieved through the coupling of two tetrapeptide subchains, which were prepared from the corresponding dipeptides. The synthesis of each dipeptide involved the use of the key building blocks methyl 4-[(*tert*-butoxycarbonyl)amino]-*N*-methyl-1*H*-pyrrole-2-carboxylate (**2**, Scheme 2) and methyl 4-[(*tert*-butoxycarbonyl)amino]-*N*-methyl-1*H*-imidazole-2-carboxylate (**4**). Removal of the Boc protecting group with acid (HCl or trifluoroacetic acid (TFA)) yielded the amine component, while generation of the carboxyl component was achieved by base hydrolysis (LiOH or NaOH). Amide bond formation was facilitated primarily with the organophosphorus reagent 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT), with the products obtained in good yield with suppressed

racemisation.^[13] The reactivity of the trichloroacetyl group (haloform reaction) was also utilised in the construction of some dipeptides,^[14] where condensation with the free amine component yielded the respective products in high yield, without the need for further purification. Initial attempts at the synthesis of **HLWC-8** began with the formation of the dipeptides **9–12**. The acid of **9** was then coupled with the free amine of **10**, by using DEPBT, after removal of the Boc group with TFA, to obtain **13** in 40% yield. Compound **14** was prepared in a similar fashion to the synthesis of **13** and was obtained in a relatively high yield of 61%. The reaction of **13** with **14** was then carried out with pentafluorophenyl diphenylphosphinate (FDPP) as the coupling agent. Final incorporation of *tert*-butyl-3-aminopropylcarbamate then gave the polyamide **16** in 40% yield. Synthesis of the platinum complex, **HLWC-8**, began with the preparation of the cation $\text{trans-[PtCl(dmf)(NH}_3)_2]^+$ in situ. Addition of the free amine of the polyamide, after OH-anion exchange, then gave the platinum complex.

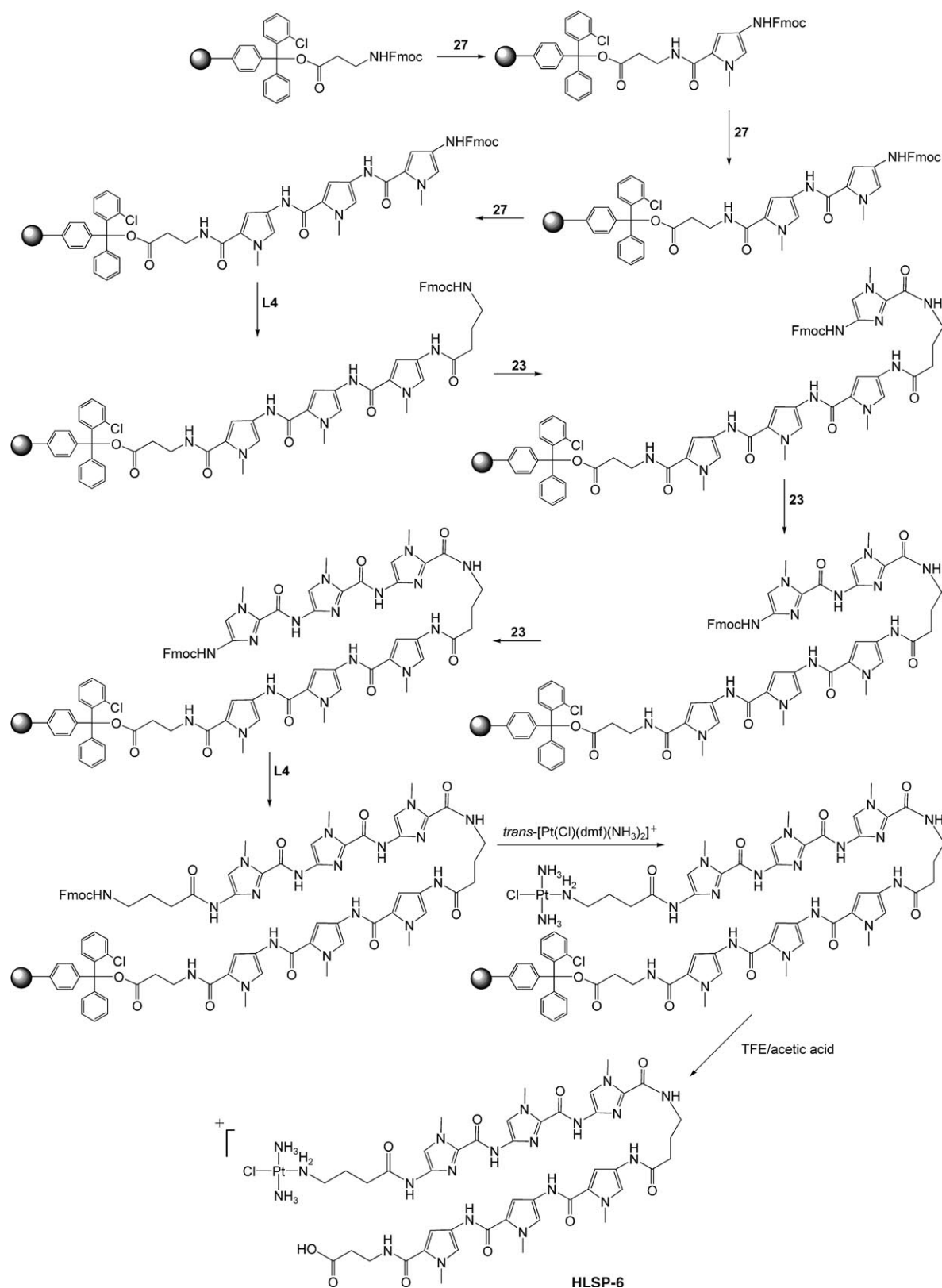
Solid-phase synthesis of HLSP-6: The solid-phase synthesis of the polyamide was initially attempted by using the Fmoc- β -alanine-OH-WANG resin (Fmoc: 9-fluorenylmethoxycarbonyl); however, despite multiple attempts, the synthesis was unsuccessful. We hypothesise that the free aromatic amines on the heterocyclic rings interact with the WANG resin to form a diketopiperazine, although characterisation of the product was not attempted. The synthesis was instead attempted by using Fmoc- β -alanine-chlorotrityl resin, which yielded the desired polyamides in high yields (Scheme 3). The chlorotrityl resin was prepared from 2-chloro-chlorotrityl resin and Fmoc- β -alanine-OH in the presence of diisopropylethylamine (DIEA).

Prior to the synthesis of **HLSP-6**, platination was attempted on single imidazole and pyrrole rings in order to optimise the reaction conditions. In the first attempt, activated transplatin ($\text{trans-[PtCl(dmf)(NH}_3)_2]^+$, 2.5 molar equiv) was treated with resin- β -alanine-pyrrole- γ -aminobutyric acid in the presence of triethylamine (TEA, 3.5 molar equiv) over a period of 20 h. The reaction afforded the product in high yield (90%) with a single ^{195}Pt NMR resonance observed at -2415 ppm. Activated transplatin was also added to resin- β -alanine-imidazole- γ -aminobutyric acid under the same conditions as those used for the pyrrole monomer. The ^{195}Pt NMR spectrum revealed the presence of two peaks at -2414 and -2436 ppm corresponding to 89 and 11% of the reaction products, respectively. The major peak corresponds to the desired product, while the minor peak is thought to correspond to a complex in which the platinum group is coordinated to the imidazole through the aromatic nitrogen atom. From these results, it appears that coordination of platinum to the amine groups is more favourable than coordination to the imidazole nitrogen atom; the latter may be eliminated by using a shorter reaction time or a smaller excess of platinum.

The monomers 4-(Fmoc-amino)-*N*-methyl-1*H*-imidazole-2-carboxylic acid (**20**), 4-(Fmoc-amino)-*N*-methyl-1*H*-pyr-



Scheme 2. Standard wet chemistry synthesis of **HLWC-8**. Boc: *tert*-butoxycarbonyl; DMF: *N,N'*-dimethylformamide.



Scheme 3. Solid-phase synthesis of **HLSP-6**. Each step (except the addition of the platinum) represents the removal of the Fmoc protecting group with 20% piperidine followed by coupling of the rings or linker.

role-2-carboxylic acid (**24**) and 4-(Fmoc-amino)butyric acid (**25**) were coupled together through a series of amide bonds to obtain **HLSP-6**. The synthesis of **HLSP-6** was achieved in 77% yield, through standard Fmoc deprotection and coupling to the activated acid. The Fmoc protecting groups were removed by using piperidine, while the acids were activated by using the coupling agent 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). *N*-methyl-2-pyrrolidone (NMP) was used to aid the dissolution of **20** and **24**. The coupling times were determined by using a ninhydrin test that monitors the disappearance of free amines from the resin. The optimal coupling time for the heterocyclic rings and linkers was found to be 3.5 h, except when the coupling included an imidazole ring in which case the amide bond formation required 10 h. Activated transplatin (1.1 molar equiv) was initially mixed with the resin in the presence of TEA for 20 h. The reaction produced a mixture of two products including the desired compound. It is believed that the other product is starting material with the free amine. A second attempt in which activated transplatin (2.5 molar equiv) was mixed with the resin in the presence of TEA for 12 h yielded the desired product. The product was cleaved from the resin by using acetic acid and trifluoroethanol (TFE).

Characterisation of the platinum hairpin complexes: HLSP-6, HLWC-8 and the intermediate compounds synthesised through wet chemistry were characterised by using ^1H NMR spectroscopy and high-resolution mass spectrometry. The platinum complexes were also characterised by ^{195}Pt NMR spectroscopy.

The characterisation of **HLSP-6** is provided here as an example of how the ^1H NMR spectroscopy and ESI-MS data were assigned for both compounds. Unfortunately, a lack of proton connectivity between the heterocyclic rings either by NOESY or g-DQCOSEY excludes the assignment of the individual proton resonances. In the spectrum of **HLSP-6**, six groups of resonances are observed, a result that is consistent with other heterocyclic hairpin molecules (Figure 1).^[15] The five resonances between 9.4–10.4 ppm integrate for six protons and correspond to the secondary amine protons. The two broad triplets at 8.21 and 7.96 ppm are the amide protons of the γ -aminobutyric acid and β -alanine residues. The nine singlet resonances between 6.6–7.6 ppm are the aromatic protons of the heterocyclic rings. The broad resonance at 5.18 ppm is that of the terminal amine protons. This resonance is approximately 0.6 ppm downfield of where the resonance is observed in the absence of platinum. The six large singlet resonances between 3.6 and 4.2 ppm correspond to the protons of the heterocyclic methyl groups. Finally, the resonances between 1.6–3.6 ppm, which integrate for 16 protons, are the CH_2 protons of the γ -aminobutyric acid and β -alanine residues.

In the ESI mass spectrum of **HLSP-6** the most prominent ion peak occurs at m/z 1259.4 (Figure 1), which corresponds to the metal complex, with the loss of the chloride counterion. The isotopic pattern of these peaks matched exactly the

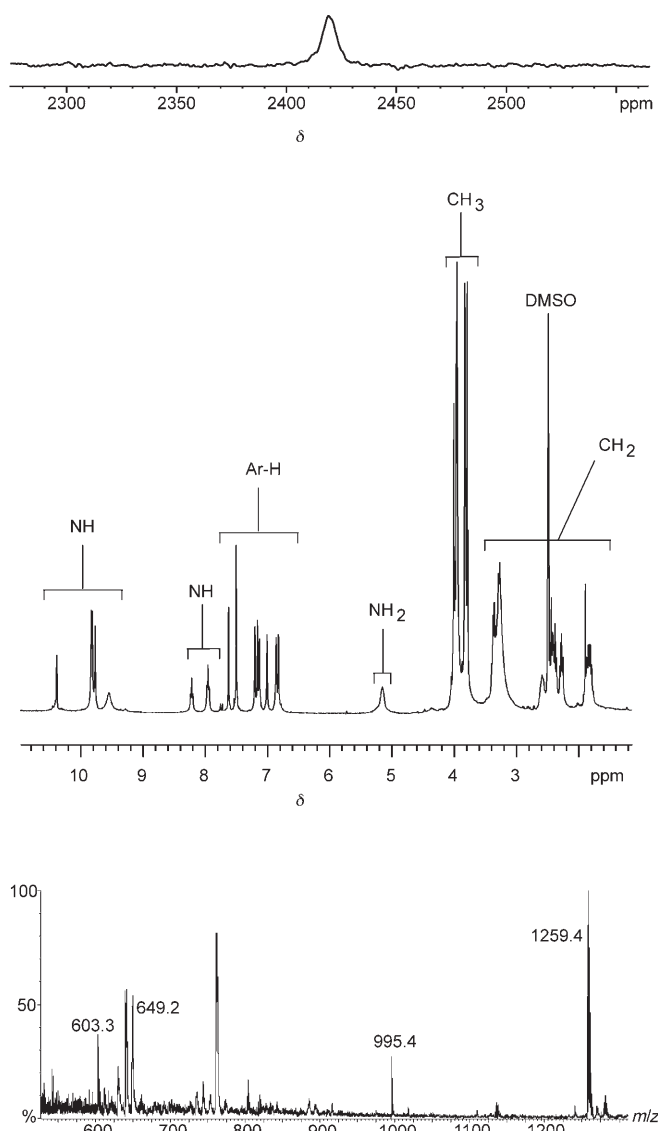


Figure 1. ^{195}Pt NMR spectrum (top), ^1H NMR spectrum (middle) and ESI mass spectrum (bottom) of **HLSP-6**.

theoretical pattern predicted for the platinum-containing hairpin. Daughter ions are also observed. The peak at m/z 603.3 is consistent with fragmentation of the parent ion $^{2+}$ minus the coordinated chloride ion and one ammine group; the peak at m/z 649.2 is consistent with fragmentation of the parent ion $^{2+}$ in which the coordinated chloride ion is displaced by dimethylsulfoxide (DMSO). Finally, the peak at m/z 995.4 is consistent with the parent ion minus the *trans*-platinum group.

In the ^{195}Pt NMR spectra, **HLWC-8** and **HLSP-6** show single platinum resonances at -2424 and -2420 ppm, respectively (Figure 1). These chemical shifts are consistent with similar metal complexes with PtN_3Cl coordination spheres, where N = two *trans*-ammine ligands and one primary amine ligand.^[16,17]

Discussion

Two hairpin polyamide platinum complexes, **HLSP-6** and **HLWC-8**, have been synthesised. **HLSP-6** contains a β -alanine residue which is selective for A or T DNA bases, three imidazole and three pyrrole rings which are selective for GGG DNA sequences and a γ -aminobutyric acid turn which is selective for A or T bases. This compound is therefore expected to bind selectively to regions of DNA that are five bases in length and comprise (A/T)GGG(A/T) sequences. Similarly, **HLWC-8** is expected to bind selectively to six base-pair regions of (A/T)CCTG(A/T) sequences. In **HLWC-8** the alkylamino linker between the polypeptide and the platinum moiety is attached to the amido group of a peptidic bond, whereas in **HLSP-6** it is attached to the carbonyl group of a peptidic bond (Scheme 1).

After analysis of the alternative synthetic methods, it was determined that solid-phase synthesis is a far more effective and efficient method for the preparation of hairpin polyamide platinum complexes. Firstly, solid-phase synthesis produces the final product in much higher yield. The total yield for the solid-phase synthesis was 77% based on the amount of initial resin, whereas the total yield for the wet synthesis was 3.8% based on the amount of initial monomer **1**. Secondly, while both methods require approximately the same number of synthetic steps (10–12), excluding the preparation of monomers, solid-phase synthesis is far more efficient with regard to time. From the attachment of the β -alanine to the chlorotriptyl resin, the total synthesis time is just three days, and the protein synthesiser can be automated, thereby removing the need for operator involvement. By contrast, wet chemistry synthesis typically takes three to four months to achieve the final polyamide complex and includes laborious purification of each intermediate before proceeding to the next step.

One advantage of wet synthesis over solid-phase synthesis is that of synthetic method development. By using wet synthesis, the desired final compounds can be attained in a variety of different synthetic combinations. In this work we chose to construct the compound from dimers to tetramers to the octamer, but we could have chosen a sequential synthetic method. In solid-phase synthesis, only sequential synthesis can be employed. Also, the compound prepared by solid-phase synthesis consists of two terminal linking groups, a β -alanine on one end of the hairpin across from a γ -aminobutyric acid on the opposing end of the hairpin. It is not known if the presence of the γ -aminobutyric acid will affect the normal β -alanine selectivity for A/T DNA bases. If it does, a longer linker such as γ -aminohexanoic acid or γ -aminooctanoic acid may be employed in the future to link the platinum centre to the hairpin. In addition, for wet chemistry, either Boc protecting groups (removed with acid) or Fmoc protecting groups (removed with base) can be employed; however, the use of acids in solid-phase synthesis will cleave the compounds from the resin prematurely, and therefore only base-labile protecting groups (or other protecting groups such as benzyl carbamate) can be used. De-

spite this, we believe that solid-phase synthesis is still the preferred method.

True DNA-binding specificity to mutant genes or telomere regions will be achieved when these compounds are able to recognise DNA sequences of between 10 and 15 base pairs.^[9] The linking of two six-ring hairpins will create a hairpin polyamide that will bind DNA with selectivity for eight to ten DNA base pairs. In addition, it is generally acknowledged that for high cytotoxicity compounds need to contain either one platinum group with two available DNA coordination sites or two platinum groups each with at least one DNA coordination site,^[2,3] although some monofunctional platinum complexes have been reported that display cytotoxicity. Attachment of two transplatin like platinum groups to the hairpin polyamides will produce multinuclear complexes that should be effective in preventing DNA transcription and replication. One downside of both **HLSP-6** and **HLWC-8** is that they are only soluble in DMSO and DMF. In DMSO, both complexes show degradation after a short period of time (≈ 30 min). The ESI mass spectrum of **HLSP-6** dissolved in DMSO shows a 95% decrease in the intensity of the parent ion peak at m/z 1259.4 after five days, while the peak at m/z 995.4, which corresponds to the parent ion minus the *trans*-platinum group, remains at around the same intensity. Several other peaks were also observed at m/z values of 595.2, 603.3, and 611.7; these correspond, respectively, to fragmentation of the parent ion²⁺ minus the coordinated chloride ion and both ammine groups, the parent ion²⁺ minus the coordinated chloride ion and one ammine group and the parent ion²⁺ minus the coordinated chloride ion.

The results of this work provide a foundation for the synthesis of hairpin polyamides with multiple platinum groups. When multiple hairpins are combined with two or more platinum groups, we predict that this will hopefully create a highly DNA-sequence-selective complex that demonstrates good water solubility (from the multiple positive charges) and possibly very high cytotoxicity.

Conclusion

We have prepared two hairpin polyamide platinum complexes by different methods. **HLWC-8**, an eight-ring polyamide, was made by standard wet chemistry techniques and **HLSP-6**, a six-ring polyamide, was made by solid-phase synthesis. The latter technique is more effective and efficient in synthesising the final compound, in terms of yield, purity and time, and is therefore the preferred method for producing these types of complexes. The results of this study provide a basis for the synthesis of more complex molecules, such as single hairpins with multiple platinum groups or multiple linked hairpins with one or more platinum groups.

Experimental Section

Materials: *N*-Methyl-1*H*-imidazole-2-carboxylic acid was purchased from Bachem. 4-(Fmoc-amino)butyric acid, (9*H*-fluoren-9-yl)methyl chloroformate and Fmoc- β -alanine were purchased from Fluka. *N*-Methyl-1*H*-imidazole, transplatin, and di-*tert*-butyl dicarbonate were purchased from the Sigma-Aldrich Chemical Company. 2-Chloro-chlorotriethyl resin and HBTU were purchased from Auspep. Boc-L-2,4-diaminobutyric acid-Fmoc was purchased from PepTech Corporation. *N*-Methyl-1*H*-pyrrole was purchased from Alfa Aesar. All other reagents and solvents were used as received unless otherwise stated. Compounds 4-nitro-2-trichloroacetyl-*N*-methyl-1*H*-pyrrole (**1**), 2, 2-trichloroacetyl-*N*-methyl-1*H*-imidazole (**3**), **4**, (Boc-amino)-*N*-methyl-1*H*-pyrrole-2-carboxylic acid (**6**), (Boc-amino)-*N*-methyl-1*H*-imidazole-2-carboxylic acid (**7**), *tert*-butyl 4-nitro-*N*-methyl-1*H*-imidazole-2-carboxylate (**21**), 4-(Fmoc-amino)-*N*-methyl-1*H*-imidazole-2-carboxylic acid (**17**), 2-trichloroacetyl-*N*-methyl-1*H*-pyrrole (**23**), *tert*-butyl 4-nitro-*N*-methyl-1*H*-pyrrole-2-carboxylate (**24**), *tert*-butyl 4-(Fmoc-amino)-*N*-methyl-1*H*-pyrrole-2-carboxylate (**25**), and 4-(Fmoc-amino)-*N*-methyl-1*H*-pyrrole-2-carboxylic acid (**18**) were synthesised as previously reported.^[18]

General methods: Reactions were monitored by thin-layer chromatography (TLC) with 0.25 mm thick pre-coated UV-sensitive silica gel plates. Compounds were visualised with short-wave ultraviolet light at 254 nm. Flash chromatography was carried out by using Kieselgel 60 (230–400) mesh. NMR spectra were recorded on either a 300 MHz Varian Mercury or 400 MHz Varian Unityplus NMR spectrometer at 35 °C, with commercially available solvents referenced to tetramethylsilane (an internal standard). ¹⁹⁵Pt NMR experiments were run on either a 400 MHz Varian Unityplus or a Bruker Advance 400 MHz NMR spectrometer externally referenced to K₂PtCl₄ ($\delta = -1631$ ppm).^[19,20] Positive-ion ESI mass spectra were acquired by using a Micromass (Wyntheshaw, UK) Quattro Micro spectrometer equipped with a Z-spray probe. Solutions containing concentrations ranging between 10–50 μ M were injected into the instruments at a flow rate of 10 μ L min⁻¹. The source and desolvation temperatures were 150 and 120 °C, respectively. The capillary tip potential and cone voltage were 2500 and 50 V, respectively. Between 10–50 acquisitions were summed to obtain the spectra, which were calibrated against a standard CsI solution (750 mm) over the same *m/z* range.

Activation of transplatin trans-[Pt(Cl)₂(NH₃)₂]: AgNO₃ (0.11 g, 0.63 mmol) was added to transplatin (0.21 g, 0.70 mmol) in DMF (4.2 mL) and the solution was stirred in the dark under N_{2(gas)} for 12 h. The mixture was passed through a 0.45 μ m cartridge filter to obtain trans-[Pt(Cl)(dmf)(NH₃)₂]⁺ in DMF.

Ethyl γ -(4-nitro-*N*-methyl-1*H*-pyrrole-2-carboxamido)butyrate (5**):** TEA (31.3 mL, 0.22 mol) was added to a cold mixture of **1** (20.00 g, 0.07 mol) and ethyl 4-aminobutyrate hydrochloride (13.68 g, 0.08 mol) in EtOAc (160 mL), and the mixture was stirred at room temperature for 1 h. The solution was diluted with CH₂Cl₂ and washed with HCl (1 M), saturated NaHCO_{3(aq)} and brine. The organic layer was dried and evaporated to dryness to yield **5** (20.77 g, 99%) as a green solid. ¹H NMR (400 MHz, [D₆]DMSO): $\delta = 8.35$ (t, 1H, *J* = 4.8 Hz), 8.07 (d, 1H, *J* = 2.0 Hz), 7.41 (d, 1H, *J* = 2.0 Hz), 4.02 (q, 2H, *J* = 7.2, 14.4 Hz), 3.89 (s, 3H), 3.21 (m, 2H), 2.32 (t, 2H, *J* = 7.6 Hz), 1.74 (m, 2H), 1.16 ppm (t, *J* = 7.2 Hz); ESI-MS: *m/z*: calcd for C₁₂H₁₈N₃O₅: 284.12; found: 284.20 [M+H]⁺.

Methyl 4-[4-(*tert*-butoxycarbonyl)amino]-*N*-methyl-1*H*-pyrrole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxylate (8**):** LiOH (1 M, 2.38 mL) was added to **2** (0.15 g, 0.60 mmol) dissolved in THF/MeOH (3:1, 8 mL), and the solution was stirred at 60 °C for 5 h. The solution was cooled and diluted with EtOAc/H₂O 1:1, then the aqueous layer was acidified with HCl (1 M, pH \approx 3). The product was extracted with EtOAc, then the organic layer was dried (anhydrous Na₂SO₄) and evaporated under reduced pressure. The brown solid was dried under vacuum for 1 h. Separately, **2** (0.12 g, 0.48 mmol) in CH₂Cl₂ (2 mL) was degassed with Ar_(gas) for 5 min. TFA/CH₂Cl₂ (1:1, 4 mL) and H₂O (0.08 mL) were added and the solution was stirred for 30 min. The solvent was removed and the residue was co-evaporated with CH₃CN to give a yellow solid, which was dried under vacuum for 1 h. The solid was dissolved in CH₂Cl₂ (5 mL), then the crude acid was added, followed by DEPBT (0.29 g, 0.95 mmol) and TEA

(0.33 mL, 2.38 mmol). The solution was stirred under Ar_(gas) for 17 h, diluted with EtOAc and washed with cold HCl (1 M), saturated NaHCO_{3(aq)} and brine. The organic layer was dried (anhydrous MgSO₄) and evaporated under reduced pressure. The residue was purified by flash chromatography (silica gel, 0.2–1% MeOH/CH₂Cl₂) to yield **8** (0.15 g, 83%) as a brown solid, which was dried under vacuum. ¹H NMR (400 MHz, [D₆]DMSO): $\delta = 9.85$ (s, 1H), 9.12 (s, 1H), 7.44 (d, 1H, *J* = 1.8 Hz), 6.88 (d, 2H, *J* = 1.8 Hz), 6.82 (s, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.72 (s, 3H), 1.44 ppm (s, 9H); ¹³C NMR (100.6 MHz, [D₆]DMSO): $\delta = 162.55$, 161.48, 158.84, 153.41, 123.20, 121.82, 121.69, 120.87, 119.78, 118.29, 108.16, 103.45, 80.28, 51.08, 36.73, 28.35 ppm; ESI-MS: *m/z*: calcd for C₁₈H₂₄N₄O₅+Na: 399.17; found: 399.00 [M+Na]⁺.

Methyl 4-(*N*-methyl-1*H*-imidazole-2-carboxamido)-*N*-methyl-1*H*-imidazole-2-carboxylate (9**):** **4** (1.00 g, 3.92 mmol) in CH₂Cl₂ (5 mL) was degassed with Ar_(gas) for 5 min. TFA/CH₂Cl₂ 1:1 (20 mL) and H₂O (0.40 mL) were added and the solution was stirred for 1 h. The solvent was removed under reduced pressure and the residue was co-evaporated with CH₃CN to give a yellow solid, which was dried under vacuum for 1 h. The solid was dissolved in EtOAc (7 mL) and TEA (1.65 mL, 11.75 mmol) was added, followed by **3** (0.89 g, 3.92 mmol). The mixture was stirred for 6 h and the resulting solid was collected, washed with EtOAc and air dried. The title compound was obtained as a yellow solid (0.72 g, 70%). ¹H NMR (400 MHz, [D₆]DMSO): $\delta = 10.07$ (s, 1H), 7.68 (s, 1H), 7.42 (s, 1H), 7.05 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.81 ppm (s, 3H); ¹³C NMR (100.6 MHz, [D₆]DMSO): $\delta = 158.79$, 156.11, 137.84, 136.18, 131.22, 127.60, 126.92, 115.49, 51.77, 35.59, 35.06 ppm; ESI-MS: *m/z*: calcd for C₁₁H₁₃N₅O₃+Na: 286.10; found: 286.07 [M+Na]⁺.

Ethyl γ -(4-[(*tert*-butoxycarbonyl)amino]-*N*-methyl-1*H*-pyrrole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxamido)butyrate (10**):** Pd/C (10%, 0.70 g) was added to a cold solution of **5** (7.14 g, 0.03 mol) in MeOH (400 mL). The mixture was stirred at room temperature under H_{2(gas)} (1 atm) for 5 h. The catalyst was removed by filtration through celite and the solvent was then removed under reduced pressure. The residue was dissolved in THF (100 mL) and added to a solution of **6** (5.51 g, 0.02 mol), DEPBT (7.25 g, 0.03 mol) and TEA (6.4 mL, 0.05 mol) in THF (200 mL) under Ar_(gas). The solution was stirred for 24 h, diluted with CH₂Cl₂ and washed with cold HCl (1 M), saturated NaHCO_{3(aq)} and brine. The organic layer was dried (anhydrous MgSO₄) and removed under reduced pressure. The residue was purified by flash chromatography (silica gel, 0.5–2% MeOH/CH₂Cl₂) to yield **10** (6.28 g, 58%) as a brown solid, which was dried under vacuum. ¹H NMR (400 MHz, [D₆]DMSO): $\delta = 9.75$ (s, 1H), 9.01 (s, 1H), 7.95 (t, 1H, *J* = 5.2 Hz), 7.14 (d, 1H, *J* = 1.6 Hz), 6.85 (d, 2H, *J* = 2.0 Hz), 6.80 (s, 1H), 4.05 (q, 2H, *J* = 7.2, 14.4 Hz), 3.79 (s, 3H), 3.78 (s, 3H), 3.19 (q, 2H, *J* = 6.0, 12.8 Hz), 2.31 (t, 2H, *J* = 7.6 Hz), 1.73 (m, 2H), 1.45 (s, 9H), 1.17 ppm (t, 3H, *J* = 7.2 Hz); ESI-MS: *m/z*: calcd for C₂₃H₃₄N₅O₆: 476.24; found: 476.3 [M+H]⁺.

Methyl 4-[4-(*tert*-butoxycarbonyl)amino]-*N*-methyl-1*H*-imidazole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxylate (11**):** Compound **2** (1.10 g, 4.32 mmol) in CH₂Cl₂ (20 mL) was degassed with Ar_(gas) for 5 min. TFA/CH₂Cl₂ 1:1 (20 mL) and H₂O (0.4 mL) were added and the solution was stirred for 1 h. The solvent was removed and the residue was co-evaporated with CH₃CN to give a yellow solid, which was dried under vacuum for 1 h. The solid was dissolved in DMF (20 mL) and **7** (1.04 g, 4.30 mmol) was added, followed by (*N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, 1.67 g, 8.71 mmol), 4-dimethylaminopyridine (DMAP, 1.32 g, 10.8 mmol) and TEA (1.81 mL, 12.9 mmol). The solution was stirred under Ar_(gas) for 17 h, diluted with EtOAc and washed with cold HCl (1 M), saturated NaHCO_{3(aq)} and brine. The organic layer was dried (anhydrous Na₂SO₄) and evaporated under reduced pressure to yield **11** (1.05 g, 64%) as a brown solid, which was dried under vacuum. ¹H NMR (400 MHz, [D₆]DMSO): $\delta = 10.06$ (s, 1H), 9.28 (s, 1H), 7.50 (s, 1H), 7.20 (s, 1H), 7.00 (s, 1H), 3.91 (s, 3H), 3.83 (s, 3H), 3.72 (s, 3H), 1.44 ppm (s, 9H); ¹³C NMR (100.6 MHz, [D₆]DMSO): $\delta = 162.76$, 161.44, 155.88, 152.63, 136.72, 133.71, 121.12, 120.75, 119.88, 112.50, 108.39, 80.91, 51.04, 36.67, 28.20 ppm; ESI-MS: *m/z*: calcd for C₁₇H₂₃N₅O₅+Na: 400.17; found: 399.95 [M+Na]⁺.

Ethyl β -(4-[4-(*tert*-butoxycarbonyl)amino]-*N*-methyl-1*H*-pyrrole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxamido)alanate (12**):** LiOH (1 M,

10.7 mL) was added to **8** (1.00 g, 2.66 mmol) dissolved in THF/MeOH 3:1 (80 mL) and the solution was stirred at 60°C for 5 h. The solution was cooled and diluted with EtOAc/H₂O 1:1. The aqueous layer was cooled and acidified with HCl (1 M, pH ≈ 3) before it was extracted with EtOAc; the organic layer was then dried (anhydrous Na₂SO₄) and evaporated under reduced pressure. The product was dried under vacuum for 1 h. The solid was dissolved in CH₂Cl₂ (70 mL) and β-alanine hydrochloride (0.82 g, 5.31 mmol) was added, followed by DEPBT (1.59 g, 5.31 mmol) and TEA (2.24 mL, 15.94 mmol). The solution was stirred under Ar_(gas) for 17 h, diluted with EtOAc and washed with cold HCl (1 M), saturated NaHCO_{3(aq)} and brine. The organic layer was dried (anhydrous Na₂SO₄) and evaporated under reduced pressure. The residue was purified by flash chromatography (silica gel, 1–2% MeOH/CH₂Cl₂) to yield **12** (1.02 g, 83%) as a brown solid, which was dried under vacuum. ¹H NMR (400 MHz, [D₆]DMSO): δ = 9.80 (s, 1H), 9.07 (s, 1H), 8.01 (t, 1H, *J* = 5.6 Hz), 7.15 (d, 1H, *J* = 1.6 Hz), 6.87 (s, 2H), 6.83 (d, 2H, *J* = 1.6 Hz), 6.80 (s, 2H), 4.06 (q, 2H, *J* = 7.5, *J* = 14.4 Hz), 3.79 (s, 3H), 3.77 (s, 3H), 3.39 (q, 2H, *J* = 5.6, *J* = 14.4 Hz), 2.51 (t, 2H, *J* = 6.8 Hz), 1.44 (s, 9H), 1.17 ppm (t, 3H, *J* = 7.2 Hz); ¹³C NMR (100.6 MHz, [D₆]DMSO): δ = 171.38, 161.30, 158.36, 152.85, 122.79, 122.63, 122.30, 122.16, 117.89, 116.98, 104.28, 103.74, 78.27, 59.88, 36.88, 36.05, 34.79, 34.02, 28.20, 14.09 ppm; ESI-MS: *m/z*: calcd for C₂₂H₃₁N₅O₆+Na: 484.23; found: 484.07 [M+Na]⁺.

Ethyl γ-(4-[4-(*N*-methyl-1*H*-imidazole-2-carboxamido)-*N*-methyl-1*H*-imidazole]-*N*-methyl-1*H*-pyrrole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxamido)butyrate (13**):** LiOH (1 M, 26.6 mL) was added to **9** (1.75 g, 0.01 mol) dissolved in THF/MeOH 3:1 (80 mL) and the solution was stirred at 60°C for 2 h. The solution was cooled, the solvent was removed and the residue was acidified with concentrated HCl (pH 2). The crude acid was dried under vacuum for 2 h. Separately, **10** (3.48 g, 0.01 mol) in CH₂Cl₂ (40 mL) was degassed with Ar_(gas) for 5 min. TFA/CH₂Cl₂ (1:1, 20 mL) and H₂O (0.8 mL) were added and the solution was stirred for 1 h. The solvent was removed and the residue was co-evaporated with CH₃CN to give a red solid, which was dried under vacuum for 1 h. The solid was then dissolved in DMF (20 mL) and the acid was added, followed by DEPBT (2.19 g, 0.01 mol) and TEA (4.7 mL, 0.03 mol). The solution was stirred under Ar_(gas) for 17 h, diluted with CHCl₃ and washed with cold H₂O, HCl (1 M), saturated NaHCO_{3(aq)} and brine. The organic layer was dried (anhydrous MgSO₄) and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel, 0.5–3% MeOH/CH₂Cl₂) to yield **13** (1.60 g, 40%) as a light brown solid. ¹H NMR (400 MHz, [D₆]DMSO): δ = 10.27 (s, 1H), 9.86 (s, 1H), 9.68 (s, 1H), 7.97 (t, 1H, *J* = 4.8 Hz), 7.56 (s, 1H), 7.44 (s, 1H), 7.27 (s, 1H), 7.17 (d, 1H, *J* = 1.6 Hz), 7.13 (d, 1H, *J* = 2.0 Hz), 7.07 (s, 1H), 6.87 (t, 1H, *J* = 6.8 Hz), 4.04 (q, 2H, *J* = 7.2, 14.4 Hz), 4.01 (s, 6H), 3.84 (s, 3H), 3.79 (s, 3H), 3.19 (q, 2H, *J* = 6.4, 13.6 Hz), 2.31 (t, 2H, *J* = 7.6 Hz), 1.74 (m, 2H), 1.17 ppm (t, 3H, *J* = 6.8 Hz); ESI-MS: *m/z*: calcd for C₂₈H₃₅N₁₀O₆: 607.27; found: 607.40 [M+H]⁺.

Compound 14: LiOH (1 M, 9.1 mL) was added to **11** (0.86 g, 2.27 mmol) dissolved in THF/MeOH 3:1 (60 mL) and the solution was stirred at 60°C for 5 h. The solution was cooled and diluted with EtOAc/H₂O 1:1. The aqueous layer was acidified with HCl (1 M, pH ≈ 3) and extracted with EtOAc, then the organic layer was dried (anhydrous Na₂SO₄) and concentrated. The product was dried under vacuum for 1 h. Separately, **12** (0.89 g, 1.93 mmol) in CH₂Cl₂ (50 mL) was degassed with Ar_(gas) for 5 min. TFA/CH₂Cl₂ 1:1 (7 mL) and H₂O (0.08 mL) were added and the solution was stirred for 1 h. The solvent was removed and the residue was co-evaporated with CH₃CN to give a red solid, which was dried under vacuum for 1 h. The solid was dissolved in DMF (80 mL) and the acid was added, followed by DEPBT (1.16 g, 3.87 mmol) and Ar_(gas) (1.63 mL, 11.60 mmol). The solution was stirred under Ar_(gas) for 36 h, diluted with CHCl₃ and washed with cold HCl (1 M), saturated NaHCO_{3(aq)} and brine. The organic layer was dried (anhydrous Na₂SO₄) and evaporated under reduced pressure. The residue was purified by flash chromatography (silica gel, 1–4% MeOH/CH₂Cl₂) to yield **14** (0.83 g, 61%) as a red solid, which was dried under vacuum. ¹H NMR (400 MHz, [D₆]DMSO): δ = 9.92 (s, 2H), 9.89 (s, 1H), 9.32 (s, 1H), 8.04 (t, 1H, *J* = 5.6 Hz), 7.27 (d, 1H, *J* = 1.2 Hz), 7.23 (d, 1H, *J* = 1.6 Hz), 7.18 (d, 2H, *J* = 1.6 Hz), 7.14 (d, 1H, *J* = 1.2 Hz), 7.04 (d, 1H, *J* = 2.0 Hz), 6.84 (d, 1H, *J* =

1.6 Hz), 4.07 (q, 2H, *J* = 7.2, 14.4 Hz), 3.93 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.79 (s, 3H), 3.40 (q, 2H, *J* = 6.4, 12.8 Hz), 2.52 (t, 2H, *J* = 7.2 Hz), 1.45 (s, 9H), 1.18 ppm (t, 3H, *J* = 7.2 Hz); ¹³C NMR (100.6 MHz, [D₆]DMSO): δ = 171.35, 162.27, 161.27, 158.42, 155.76, 136.34, 134.14, 123.00, 122.74, 122.64, 122.15, 122.08, 121.18, 118.61, 118.44, 117.91, 113.72, 104.87, 104.70, 104.27, 78.97, 59.85, 36.13, 36.05, 35.91, 35.75, 34.92, 34.78, 34.01, 30.73, 28.08, 14.07 ppm; ESI-MS: *m/z*: calcd for C₃₃H₄₂N₁₀O₈+Na: 729.32; found: 729.17 [M+Na]⁺.

Compound 15: NaOH (0.6 M, 5.8 mL) was added to **13** (0.70 g, 1.15 mmol) in EtOH (30 mL) and the mixture was stirred overnight. The mixture was diluted with EtOAc/H₂O 1:1, then the aqueous layer was acidified with concentrated HCl (pH 2) and extracted with CHCl₃. The organic layer was dried (anhydrous MgSO₄) and evaporated under reduced pressure. The crude acid was dried under vacuum for 1 h. Separately, 4 M HCl/EtOAc (2 mL) was added to **14** (0.57 g, 0.81 mmol) in EtOAc (5 mL) and the solution was stirred under Ar_(gas) for 30 min. The solvent was removed and the residue was co-evaporated with CH₃CN to give a yellow solid, which was dried under vacuum for 1 h. The solid was dissolved in DMF (20 mL) and the acid was added, followed by pentafluorophenyl diphenylphosphinate (FDPP, 0.93 g, 2.44 mmol) and DIEA (0.67 mL, 4.1 mmol). The solution was stirred under Ar_(gas) for 36 h, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, 1–10% MeOH/CH₂Cl₂) to yield **15** (0.42 g, 42%) as a brown solid, which was dried under vacuum. ¹H NMR (400 MHz, [D₆]DMSO): δ = 10.33 (s, 1H), 10.25 (s, 1H), 9.96 (s, 1H), 9.93 (s, 1H), 9.92 (s, 1H), 9.88 (s, 1H), 9.70 (s, 1H), 8.03 (t, 1H, *J* = 5.6 Hz), 7.56 (s, 1H), 7.46 (d, 1H, *J* = 2.4 Hz), 7.27 (s, 4H), 7.23 (d, 1H, *J* = 1.6 Hz), 7.17 (d, 2H, *J* = 2.0 Hz), 7.14 (s, 2H), 7.07 (d, 1H, *J* = 1.6 Hz), 7.04 (d, 1H, *J* = 1.6 Hz), 6.90 (d, 1H, *J* = 1.6 Hz), 6.84 (d, 1H, *J* = 1.6 Hz), 4.07 (q, 2H, *J* = 7.2, 14.0 Hz), 4.00 (s, 6H), 3.95 (s, 3H), 3.85 (s, 3H), 3.84 (s, 6H), 3.80 (s, 3H), 3.79 (s, 3H), 2.66 (m, 2H), 2.36 (m, 2H), 2.17 (t, 2H, *J* = 8.4 Hz), 1.90 (m, 2H), 1.79 (t, 2H, *J* = 6.8 Hz), 1.18 ppm (t, 3H, *J* = 7.2 Hz); ESI-MS: *m/z*: calcd for C₅₄H₆₃N₂₀O₁₁: 1167.49; found: 1167.70 [M+H]⁺.

Compound 16: NaOH (0.6 M, 1.8 mL) was added to **15** (0.42 g, 0.36 mmol) in EtOH (16 mL) and the mixture was stirred overnight. The solvent was removed under reduced pressure, then the residue was acidified with HCl (1 M, pH ≈ 3) and co-evaporated with CH₃CN. The yellow solid was dried under vacuum for 2 h and dissolved in DMF (7 mL), then *tert*-butyl 3-aminopropylcarbamate (0.11 g, 0.61 mmol) was added, followed by FDPP (0.42 g, 1.08 mmol) and DIEA (0.18 mL, 1.08 mmol). The mixture was stirred under Ar_(gas) for 36 h, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, 2–10% MeOH/CH₂Cl₂) to yield **16** (0.23 g, 49%) as a brown solid, which was dried under vacuum. ¹H NMR (400 MHz, [D₂]DMF): δ = 10.37 (s, 1H), 10.35 (s, 1H), 10.09 (s, 2H), 10.02 (s, 1H), 9.56 (s, 1H), 8.12 (s, 1H), 7.95 (s, 1H), 7.57 (s, 1H), 7.50 (s, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 7.37 (s, 1H), 7.34 (s, 1H), 7.31 (s, 4H), 7.21 (s, 1H), 7.18 (s, 2H), 7.10 (s, 1H), 7.07 (s, 1H), 6.98 (s, 1H), 6.68 (s, 1H), 4.12 (s, 3H), 4.10 (s, 3H), 4.06 (s, 3H), 3.98 (s, 3H), 3.97 (s, 3H), 3.94 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.51 (m, 2H), 3.37 (m, 2H), 3.20 (m, 2H), 3.07 (m, 2H), 2.51 (t, 2H, *J* = 7.2 Hz), 2.46 (t, 2H, *J* = 7.2 Hz), 1.93 (t, 2H, *J* = 6.4 Hz), 1.61 (t, 2H, *J* = 6.4 Hz), 1.38 ppm (s, 9H); ESI-MS: *m/z*: calcd for C₆₀H₇₅N₂₂O₁₂: 1296.37; found: 1296.90 [M+H]⁺.

trans-Chlorodiamino[3-(propanamido)amino-3-[4-[4-(4-[4-(4-[4-(*N*-methyl-1*H*-imidazole-2-carboxamido)-*N*-methyl-1*H*-imidazole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxamido)-butyrylamino]-*N*-methyl-1*H*-imidazole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxamido)-*N*-methyl-1*H*-pyrrole-2-carboxamido]platinum(II) nitrate (HLWC-8**):** 4 M HCl/EtOAc (0.25 mL) was added to **16** (0.05 g, 0.04 mmol) and the mixture was stirred for 30 min. The yellow solid was co-evaporated with CH₃CN and dried under vacuum. The solid was dissolved in MeOH and stirred with DOWEX 550A OH-anion exchange resin for 1 h. The solution was decanted and the solvent was removed under reduced pressure. The solid was dried under vacuum. Activated transplatin (0.007 g, 0.02 mmol) in DMF (1.5 mL) was added to **16** in DMF (3 mL) and the solution was stirred in the dark overnight, before the solvent was re-

moved under reduced pressure. Acetone was added and the mixture was cooled at 4 °C overnight. The resultant orange precipitate (0.029 g, 80 %) was collected and dried under vacuum. ¹H NMR (400 MHz, [D₂]DMF): δ = 10.38 (s, 1H), 10.33 (s, 1H), 10.09 (s, 2H), 10.07 (s, 1H), 9.56 (s, 1H), 8.33 (s, 1H), 8.11 (s, 1H), 8.06 (s, 1H), 7.58 (s, 1H), 7.50 (s, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 7.37 (s, 1H), 7.34 (s, 2H), 7.31 (s, 4H), 7.23 (s, 1H), 7.19 (s, 2H), 7.10 (s, 1H), 7.07 (s, 1H), 7.03 (s, 1H), 4.12 (s, 3H), 4.10 (s, 3H), 4.06 (s, 3H), 3.98 (s, 3H), 3.97 (s, 3H), 3.94 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.70 (s, 6H), 3.37 (m, 2H), 3.32 (m, 2H), 3.07 (m, 2H), 2.94 (m, 2H), 2.77 (m, 2H), 2.51 (t, 2H, *J* = 7.6 Hz), 1.94 (t, 2H, *J* = 6.4 Hz), 1.27 ppm (m, 2H); ¹⁹⁵Pt NMR (85 MHz, [D₂]DMF): δ = -2424 ppm.

Solid-phase synthesis

Automated protocols: The automated synthesis was performed on a Symphony Quartet 3.21 protein synthesiser on a 0.28 mmol scale. Each cycle of monomer addition involved a CH₂Cl₂ wash, a DMF wash, deprotection with 20 % piperidine/DMF for 3 min, draining of the reaction vessel, a DMF wash, deprotection for 17 min, a CH₂Cl₂ wash, two DMF washes, draining of the reaction vessel, coupling for 3.5 h (10 h when coupling to an imidazole ring), addition of DMSO (1 mL), coupling for 1 h, and finally draining of the reaction vessel. The activated transplatin was coupled to the resin in the presence of TEA (0.137 mL, 0.98 mmol) for 12 h in the dark. The resin was washed with DMF (1 ×), brine (2 ×), H₂O (2 ×), DMF (2 ×), and CH₂Cl₂ (3 ×), then dried for 1 h. The activated acids were added manually to the reaction vessel at the end of every deprotection cycle. The cycle was interrupted, the reaction vessel was vented, the activated acid was added and the cycle was resumed.

Fmoc-β-alanine-chlorotrityl resin: 2-Chloro-chlorotrityl resin (0.50 g, 0.5 mmol) was suspended in anhydrous CH₂Cl₂ (5 mL). Separately, Fmoc-β-alanine-OH (0.16 g, 0.5 mmol) was stirred for 5 min in anhydrous CH₂Cl₂ (4 mL) and DIEA (0.26 g, 0.348 mL, 2 mmol). The Fmoc-β-alanine-OH solution was added to the resin mixture and shaken for 5 h. MeOH (2.5 mL) was added and the mixture was shaken for 30 min. The resin was filtered and washed with CH₂Cl₂, then the organic layer was dried. A yield of 0.60 g (0.622 mmol g⁻¹) was obtained.

4-Nitro-2-trichloroacetyl-N-methyl-1H-imidazole (17): Fuming HNO₃ (14 mL, 0.3 mol) and then H₂SO₄ (0.56 mL) were added to acetic anhydride (160 mL) at -5 °C. **3** (20.00 g, 0.09% mol) was added slowly over a period of 1 h. The solution was allowed to warm to room temperature and stirred overnight. CHCl₃ (180 mL) was added and the solution was extracted with NaHCO₃ (3 × 50 mL, 1 M) and brine (3 × 50 mL). The organic layers were dried with anhydrous MgSO₄ and evaporated under reduced pressure to obtain a red/brown oil. The oil was mixed with hexane/EtOAc 1:1 (24 mL) and sonicated. The mixture was left at -20 °C for 1 h and the precipitate was filtered to obtain **17** (10.32 g, 64.4%) as a yellow powder. M.p. 139–141 °C; ¹H NMR (300 MHz, [D₆]DMSO): δ = 8.82 (s, 1H), 4.06 ppm (s, 3H).

tert-Butyl 4-[(9-fluorenylmethoxycarbonyl)amino]-N-methyl-1H-imidazole-2-carboxylate (19): Compound **18** (2.00 g, 8.81 mmol) was dissolved in DMF (40 mL) and 10 % Pd/C (0.2 g) was added. The mixture was shaken for 20 h under H₂(gas) (34 psi) and then filtered through Celite and rinsed with DMF (80 mL). Fmoc-Cl (2.22 g, 8.60 mmol) and NaHCO_{3(aq)} (0.72 g, 8.60 mmol) were added and the suspension was stirred overnight under N₂(gas). The base was filtered and (CH₃CH₂)₂O (60 mL) was added. The diethyl ether layer was extracted with H₂O (10 mL), brine (10 mL) and H₂O (10 mL). The product was then dried with anhydrous MgSO₄ and filtered, before the solvent was removed under vacuum to yield green oil. The oil was purified by flash chromatography (silica gel, hexane/EtOAc (3:1), R_f = 0.16) to yield **19** as a white solid (1.66 g, 45.2%). M.p. 105–107 °C; ¹H NMR (300 MHz, [D₆]DMSO): δ = 10.39 (s, 1H), 7.89 (d, 2H, *J* = 7.4 Hz), 7.65 (d, 2H, *J* = 7.6 Hz), 7.41 (t, 2H, *J* = 7.0 Hz), 7.30 (t, 2H, *J* = 7.5 Hz), 7.06 (s, 1H), 4.40 (d, 2H, *J* = 6.9 Hz), 4.28 (t, 1H, *J* = 6.9 Hz), 3.85 (s, 3H), 1.58 ppm (s, 9H).

Activation of Fmoc-pyrrole acid: Compound **24** (0.23 g, 0.62 mmol) was dissolved in DMF (2 mL) and NMP (5 mL). HBTU (0.212 g, 0.56 mmol) was added, followed by DIEA (0.31 mL, 1.78 mmol), and the solution was stirred for 10 min.

Activation of Fmoc-imidazole acid: Compound **20** (0.26 g, 0.62 mmol) was dissolved in DMF (4 mL) and NMP (4 mL). HBTU (0.212 g,

0.56 mmol) was added, followed by DIEA (0.31 mL, 1.78 mmol), and the solution was stirred for 10 min.

Activation of 4-(Fmoc-amino)butyric acid: Compound **25** (0.20 g, 0.62 mmol) was dissolved in DMF (5 mL). HBTU (0.21 g, 0.56 mmol) was added, followed by DIEA (0.31 mL, 1.78 mmol), and the solution was stirred for 10 min.

trans-Chlorodiammino-[4-(butyrylamido)amino-4-[[2-(2-[4-(2-[2-[3-(propanamido)-N-methyl-1H-pyrrole-2-carboxamido]-N-methyl-1H-pyrrole-2-carboxamido]-N-methyl-1H-pyrrole-2-carboxamido)-butyrylamino]-N-methyl-1H-imidazole-2-carboxamido]-N-methyl-1H-imidazole-2-carboxamido]]]platinum(II) nitrate (HLSP-6): Compound **HLSP-6** was prepared by automated synthesis protocols. Once synthesised, it was cleaved from the resin by addition of a solution of CH₂Cl₂ (4.2 mL), TFE (1.2 mL) and CH₃COOH (0.6 mL) to the resin before the mixture was shaken very gently for 1.5 h. The resin was removed by filtration and washed with TFE/CH₂Cl₂ 1:4 (6 mL). The yellow/brown filtrate was collected and the solvent was reduced in volume under pressure to ≈ 2 mL. Cold diethyl ether (4 mL) was added and the mixture left in the fridge for 1.5 h. The solvent was decanted and water (3 mL) was added to the solid, before the solution was lyophilised to yield **HLSP-6** (0.27 g, 77.1%) as a brown/yellow solid. ¹H NMR (300 MHz, [D₆]DMSO): δ = 10.38 (s, 1H), 9.83 (s, 1H), 9.81 (s, 1H), 9.76 (s, 1H), 9.55 (br, 2H), 8.21 (t, 1H, *J* = 5.8 Hz), 7.96 (t, 1H, *J* = 5.4 Hz), 7.63 (s, 1H), 7.51 (s, 2H), 7.21 (d, 1H, *J* = 1.4 Hz), 7.17 (d, 1H, *J* = 1.4 Hz), 7.15 (d, 1H, *J* = 1.4 Hz), 7.01 (d, 1H, *J* = 1.4 Hz), 6.86 (d, 1H, *J* = 1.4 Hz), 6.83 (d, 1H, *J* = 1.6 Hz), 5.18 (t, 2H), 4.03 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.33 (m, 6H), 2.47 (t, 2H, *J* = 7.1 Hz), 2.38 (t, 2H, *J* = 7.5 Hz), 2.29 (t, 2H, *J* = 7.4 Hz), 1.82 ppm (m, 4H, *J* = 7.1 Hz); ¹⁹⁵Pt NMR (85 MHz, [D₆]DMSO): δ = -2420 (brs); ESI-MS: *m/z*: calcd for C₄₄H₆₀ClN₂₀O₁₀Pt: 1259.4; found: 1259.4 [*M*-Cl⁻].

Acknowledgements

We thank Dr. Chris Chandler from Auspep for his help in solid-phase synthesis and the University of Western Sydney for providing scholarships, grants and funding for the protein synthesiser. Special thanks go to Craig Brodie, Warren Howard and Marc van Holst for their continuous support and help.

- [1] E. Wong, C. M. Giandomenico, *Chem. Rev.* **1999**, *99*, 2451.
- [2] N. J. Wheate, J. G. Collins, *Coord. Chem. Rev.* **2003**, *241*, 133.
- [3] N. J. Wheate, J. G. Collins, *Curr. Med. Chem. Anti-Cancer Agents* **2005**, *5*, 267.
- [4] M. S. Bali, D. P. Buck, A. J. Coe, A. I. Day, J. G. Collins, *Dalton Trans.* **2006**, 5337.
- [5] N. J. Wheate, A. I. Day, R. J. Blanch, A. P. Arnold, C. Cullinane, J. G. Collins, *Chem. Commun.* **2004**, 1424.
- [6] D. I. Jodrell, T. R. J. Evans, W. Steward, D. Cameron, J. Prendiville, C. Aschele, C. Noberasco, M. Lind, J. Carmichael, N. Dobbs, G. Camboni, B. Gatti, F. De Braud, *Eur. J. Cancer* **2004**, *40*, 1872.
- [7] T. A. Hensing, N. H. Hanna, H. H. Gillenwater, M. G. Camboni, C. Allievi, M. A. Socinski, *Anti-Cancer Drugs* **2006**, *17*, 697.
- [8] D. Jaramillo, N. J. Wheate, S. F. Ralph, W. A. Howard, Y. Tor, J. R. Aldrich-Wright, *Inorg. Chem.* **2006**, *45*, 6004.
- [9] P. B. Dervan, *Bioorg. Med. Chem.* **2001**, *9*, 2215.
- [10] J. W. Trauger, E. E. Baird, P. B. Dervan, *Nature* **1996**, *382*, 559.
- [11] R. P. L. deClairac, B. H. Geierstanger, M. Mrksich, P. B. Dervan, D. E. Wemmer, *J. Am. Chem. Soc.* **1997**, *119*, 7909.
- [12] S. White, E. E. Baird, P. B. Dervan, *J. Am. Chem. Soc.* **1997**, *119*, 8756.
- [13] H. Li, X. Jiang, H. Ye, C. Fan, T. Romoff, M. Goodman, *Org. Lett.* **1999**, *1*, 91.
- [14] E. Nishiwaki, S. Tanaka, H. Lee, M. Shibuya, *Heterocycles* **1988**, *27*, 1945.

- [15] U. Ellervik, C. C. Wang, P. B. Dervan, *J. Am. Chem. Soc.* **2000**, *122*, 9354.
- [16] H. Rauter, R. Di Domenico, E. Menta, A. Oliva, Y. Qu, N. Farrell, *Inorg. Chem.* **1997**, *36*, 3919.
- [17] M. E. Oehlsen, Y. Qu, N. Farrell, *Inorg. Chem.* **2003**, *42*, 5498.
- [18] D. Jaramillo, Q. Liu, J. Aldrich-Wright, Y. Tor, *J. Org. Chem.* **2004**, *69*, 8151.
- [19] N. J. Wheate, B. J. Evison, A. J. Herlt, D. R. Phillips, J. G. Collins, *Dalton Trans.* **2003**, 3486.
- [20] N. J. Wheate, C. Cullinane, L. K. Webster, J. G. Collins, *Anti-Cancer Drug Des.* **2001**, *16*, 91.

Received: October 19, 2006
Published online: January 17, 2007