PAPER

NJC www.rsc.org/njc

Solid-phase synthesis of peptide-platinum complexes using platinum-chelating building blocks derived from amino acids

Marc S. Robillard, Sophie van Alphen, Nico J. Meeuwenoord, Bart A. J. Jansen, Gijs A. van der Marel, Jacques H. van Boom† and Jan Reedijk*

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands. E-mail: reedijk@chem.leidenuniv.nl; Fax: +31-71-527-4671; Tel: +31-71-527-4459

Received (in Montpellier, France) 21st July 2004, Accepted 17th September 2004
First published as an Advance Article on the web 8th December 2004

Amino acids have been employed as precursors in the synthesis of platinum-chelating solid-phase building blocks. These chelating molecules were subsequently successfully used in the solid-phase synthesis of peptide-platinum complexes. The newly introduced functionality in the chelating part, as well as the nature of the pendant peptide, was shown to have an important influence on the anticancer activity of the complexes.

DOI: 10.1039/b411219a

Introduction

The use of combinatorial chemistry complemented by high-throughput screening has developed into a well-established protocol in drug discovery. The development of an efficient synthetic procedure for platinum complexes would significantly facilitate the implementation of such a protocol in the search for novel anticancer platinum drugs. In this respect, we have demonstrated the compatibility of solid-phase synthetic strategies with platinum chemistry by the synthesis of a variety of cisplatin-like complexes and even dinuclear platinum complexes.[1] Intrigued by the finding that amino acid residues and peptides can be successfully employed as site-specific DNA-interacting elements conjugated to metal complexes,[2] we recently constructed a library of dichloroplatinum(II) tripeptide complexes, **1**, by an automated parallel solid-phase synthesis protocol (Fig. 1).[1c]

Dichloroplatinum(II) tripeptide complexes **1** were designed on the basis of the assumption that the use of dipeptides **B** conjugated to a dichloroplatinum moiety **A** (from building block **3**) would not only improve targeting to the DNA but also provide specific, possibly favorable additional interactions with the platinated DNA.[2–4] The modular nature of structure **1** allowed for the facile introduction of a high degree of diversity by varying the amino acids in the glycine-tethered dipeptide (**B**).

Inspection of the structure of complexes **1** indicates that introduction of additional functionalities in the platinum chelating building block (**A**) itself would give complexes **2**, thereby further increasing the diversity of accessible platinum structures. An appealing strategy therefore involves the construction of analogs of the platinum-chelating ethylenediamine derivative **3** (Fig. 1) from amino acids as versatile key building blocks to give ligands **4**. Incorporation of **4** to give **2** would lead to the introduction of chiral centers near to the platinum complex and potential DNA-interacting moieties close to the DNA platination site. In this paper we report the preparation of platinum-chelating building blocks **4** (see Fig. 1) and their application in the solid-phase assembly of tripeptide-platinum complexes **2**. The effect of the functionalities close to the platinum core, as well as the appended functionalities (*e.g.* arginine), on the anticancer properties of the complexes was investigated.

Results and discussion

The solid-phase assembly of the projected platinum complexes **2** commences with the solution-phase synthesis of platinum-chelating building block **4**, using amino acids as starting compounds. One approach to the synthesis of target compounds **4** (Scheme 1) comprises the reductive amination of Boc–aminoaldehyde (**6**) with amino acids **5**,[5] and subsequent protective group manipulation, thereby yielding target compounds **4**. Reductive amination of Boc–aminoethanal **6** with *tert*-butyl protected alanine (**5a**) or phenylalanine (**5b**), using $NaBH_3CN$ as reducing agent, gave *tert*-butyl esters **7a,b**, respectively. The secondary nitrogen in **7a,b** was protected with a Fmoc group, affording compounds **8a,b**. Subsequent acidolysis of the Boc and *tert*-butyl groups, followed by Fmoc protection of the primary amine in **9a,b**, resulted in bis-Fmoc protected building blocks **4a** and **4b**.

An alternative approach[6] uses unprotected amino acids (Scheme 1). Reductive amination of a suspension of phenylalanine (**10b**) in methanol afforded insoluble **11b**. Dissolution of **11b** in dichloromethane was mediated by *in situ* silylation of the carboxylic acid and the secondary amine by bis (trimethylsilyl)acetamide.[7] Subsequently, the secondary amine was Fmoc-protected, giving **12b**. Acidolysis of the Boc group, providing **9b**, and Fmoc-protection of the resulting primary amine, furnished the phenylalanine-based building block **4b** in an improved overall yield compared to the approach starting from **5**.

To investigate the effect on anticancer activity of an appended potential DNA-binding guanidinium function, as well as methyl and benzyl moieties near to the platinum core, the obtained building blocks **4a** and **4b** and unfunctionalized **3** (R^3 = H) were applied in the solid phase construction of peptide-platinum complexes **18a–f** (Scheme 2). To this end, immobilized dipeptides glycine–glycine and glycine–arginine (**14**), prepared *via* a standard peptide synthesis protocol, were elongated with building blocks **3** and **4a,b** to furnish the six immobilized tripeptides **15a–f** (Scheme 2). The incorporation of the two new building blocks **4a** and **4b** proceeded successfully, as ascertained by determining the loading of **15d** and **15f**.

† Deceased July 31, 2004.

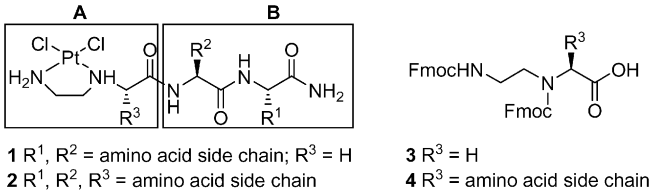
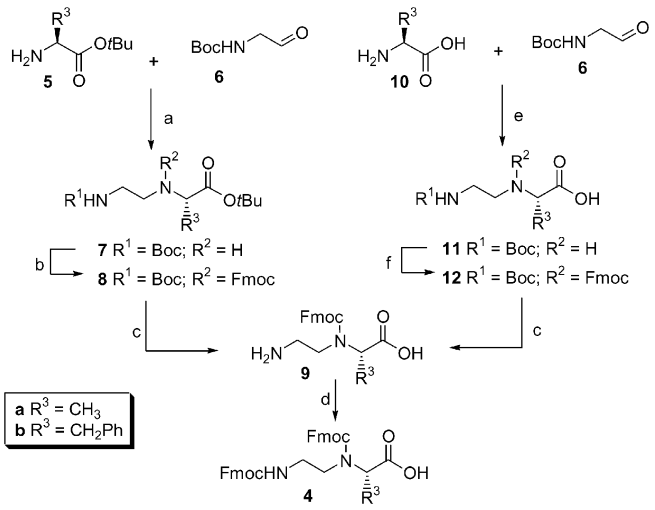
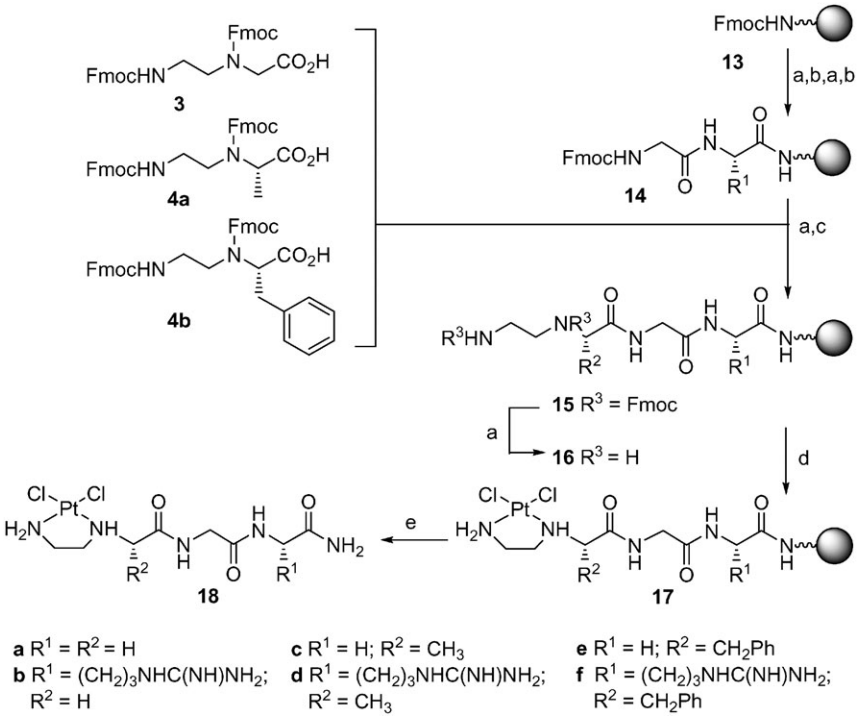


Fig. 1 Peptide-platinum complexes **1** and **2** and platinum-chelating building blocks **3** and **4**.

Unmasking of the ethylenediamine moiety in **15**, followed by treatment with K_2PtCl_4 (~6 eq.) in DMF–H_2O (9 : 1) for 48 h in the dark, provided immobilized dichloroplatinum complexes **17a–f**. Treatment of **17a–f** with TFA–H_2O (95 : 5) resulted in removal of the arginine-protecting Pbf group and concomitant cleavage from the solid support, yielding crude **18a–f**. Purification of these complexes with gel permeation chromatography (HW-40, 1% aq. AcOH) was troublesome,



Scheme 1 Synthesis of alanine- and phenylalanine-derived building blocks **4a** and **4b**. Reagents and conditions: (a) 1.3 equiv. $NaBH_3CN$, 3% AcOH in MeOH; (b) 2 equiv. DiPEA, 1.1 equiv. Fmoc–OSu, DCM; (c) DCM–TFA (1 : 1); (d) 3 equiv. DiPEA, 1.1 equiv. Fmoc–OSu, dioxane; (e) 1.1 equiv. $NaBH_3CN$, MeOH; (f) 1 equiv. DiPEA, 1.75 equiv. BTSA, DCM, 1.1 equiv. Fmoc–Osu.



Scheme 2 Solid-phase synthesis of peptide-platinum complexes **18a–f**. Reagents and conditions: (a) piperidine; (b) Fmoc–AA–OH, BOP, HOBt, DiPEA, NMP; (c) BOP, HOBt, DiPEA, NMP; (d) K_2PtCl_4, DMF–H_2O (9 : 1); e) TFA–H_2O (19 : 1).

Table 1 IC_{50} values (μM) determined in A2780 and A2780cisR cell lines using the MTT assay[8]

Complex	A2780	A2780cisR
18a	24.1	203
18b	131	≫152
18c	123	≫195
18d	136	≫154
18e	186	≫170
18f	31.8	105
Cisplatin	0.37	2.40

due to unwanted adsorption onto the column material. In response to this, the elution solvent was changed to 1% HCl in MeOH–H_2O, which countered this effect and furnished homogeneous complexes **18a–f** in 14–58% yield. The identity of tripeptide-platinum complexes **18a–f** was fully ascertained by ^{195}Pt NMR, ^{1}H NMR and mass spectroscopy. In this respect it is of interest that **18b–f** occur as diastereomers, due to the chiral coordinating nitrogen. The diastereomers of complexes **18c–f** could be observed separately with LCMS (**18c–e**), ^{1}H NMR (**18c** and **18d**) and ^{195}Pt NMR (**18d** and **18f**). However, ^{195}Pt and ^{1}H NMR failed to detect separate diastereomers of complex **18b** and insufficient column retention precluded further investigation of **18b** with LCMS. The marked difference between **18b** and **18c–f** can be explained by the lack of the additional chiral center in the platinum chelating part of **18b**. Thus, the chiral coordinating nitrogen in **18b** is too far removed from the next chiral center in the molecule, the αCH of arginine, to significantly impose diastereomeric characteristics.

Having complexes **18a–f** in hand, the cytotoxicity of each of them was evaluated in the human ovarian carcinoma A2780 (cisplatin sensitive) and A2780cisR (cisplatin resistant) cell lines. IC_{50} is defined as the concentration of the drug that results in 50% growth inhibition. The cytotoxicity results are summarized in Table 1.

These results reveal that the peptide-platinum complexes **18a–f** have a reduced activity compared to cisplatin and are also cross-resistant with cisplatin. Unfunctionalized **18a** displayed the highest activity, closely followed by phenyalanine-derived and arginine-tethered **18f**. Complexes **18b–d** have similar activities and **18e** was shown to be the least potent. Even though the pendant dipeptide, as well as the incorporated amino acid in the chelate backbone, seem to have a tremendous effect on the anticancer activity of these platinum complexes, the determined sequence of activity of this small set of peptide-platinum complexes does not yet allow to draw a clear structure-activity relationship.

Concluding remarks

The results described in this paper present a straightforward method for the synthesis of platinum-chelating building blocks from amino acids. The synthetic strategy is likely to be amendable to the incorporation of a wide range of amino acid residues in various positions on the backbone of the platinum chelating unit. Such modifications in the platinum complex impart structural diversity, which is required to expand the collection of distinct types of platinum structures available through a solid-phase combinatorial or parallel synthesis approach. Chelating ligands **4a** and **4b** were successfully used in the solid-phase synthesis of a range of peptide-platinum complexes. The newly introduced functionality in the chelating part, as well as the nature of the pendant peptide, was shown to influence the anticancer activity of the complexes. Future studies will deal with larger groups of molecules, to allow the determination of a more quantitative structure-activity relationship.

Experimental

Methods and materials

^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker DPX 300 spectrometer. Chemical shifts (δ) are given in ppm relative to TMS as an internal standard. ^{195}Pt spectra were taken on a Bruker DPX 300 spectrometer and were calibrated using K_2PtCl_4 as an external reference at $\delta = -1614$ ppm. Electrospray mass spectra were recorded on a Finnigan MAT TSQ-70 equipped with a custom-made electrospray ionization (ESI) interface. LCMS analysis was performed on a Jasco HPLC system (detection simultaneously at 214 and 254 nm) coupled to a Perkin Elmer Sciex API 165 mass instrument. An analytical Econosphere® C_{18} column (AllTech, 4.6 mm × 250 mm, 5 μm particle size) was used. The applied eluents were (A) H_2O; (B) CH_3CN and (C) 0.5% aq. TFA. Gel permeation chromatography was performed using a Fractogel HW-40 column (26 mm × 60 cm). The flow speed was 1.5 ml min^{-1}. The applied eluents were 1% aq. AcOH or 0.01 M HCl in H_2O–MeOH (1 : 1 v/v). Detection was performed at 214 nm.

All solvents and reagents were obtained from commercial sources and were used as received unless stated otherwise. DCM (Baker, p.a.) and DiPEA (Biosolve) were dried by refluxing over CaH_2 (5 g l^{-1}) for 5 h, then distilled and stored over molecular sieves (4 Å). MeOH (Rathburn, HPLC grade) was stored over 3 Å molecular sieves.

TLC analysis was conducted on DC Fertigfolien (Schleicher & Schuell, F 1500, LS 254). Compounds were visualized by UV light and by spraying with a ninhydrin solution followed by charring. Eluents for column chromatography were of technical grade and distilled before use. Column chromatography was performed on Baker silica gel 60 (0.063–0.200 mm). All reactions were performed at room temperature unless stated otherwise.

General procedure for solid-phase synthesis of peptides

Peptides were synthesized on an ABI 433A (Applied Biosystems, division of Perkin Elmer) peptide synthesizer employing a FastMoc® peptide synthesis protocol. Generally, after cleavage of the Fmoc group from the resin with piperidine, a five-fold excess of amino acid was dissolved in NMP and activated by sequential addition of 1 equiv. BOP–HOBt (0.5 M BOP and 0.5 M HOBt in DMF–NMP 1 : 1 v/v), and 2 equiv. DiPEA (1.25 M in NMP). The resulting solution was transferred to the reaction vessel, which was then shaken for 1 h. The coupling procedure of the first amino acid was performed twice to ensure a high initial loading. All solvents used in the automated peptide synthesis were of peptide synthesis grade and were purchased from Biosolve. Piperidine, DiPEA and TFA were from Biosolve. BOP and HOBt were from Neosystem Laboratoire (France). Rink Amide MBHA resin [4-(2′,4′-dimethoxyphenyl–Fmoc–aminomethyl) phenoxyacetamidonorleucyl–MBHA; 0.5 mmol g^{-1}] was purchased from NovaBiochem (Switzerland). The standard Fmoc–amino acids used in the synthesis were Fmoc–Arg(Pbf)–OH and Fmoc–Gly–OH.

***N*$^{\alpha}$-[*N*-(*tert*-Butyloxycarbonyl)-2-aminoethyl]alanine *tert*-butyl ester (7a).** Aldehyde **6** (1 g, 6.28 mmol) and H–Ala–O*t*Bu · HCl (**5a**; 1.14 g, 6.28 mmol) were dissolved in 3% AcOH in MeOH (15.7 ml) containing molecular sieves (3 Å). $NaBH_3CN$ (0.53 g, 8.48 mmol) was slowly added; the reaction mixture was stirred overnight. EtOAc (125 ml) and 5% aq. $NaHCO_3$ (50 ml) were added and the aqueous layer was extracted with EtOAc (3 × 60 ml). The combined organic layers were washed with 5% aq. $NaHCO_3$ (50 ml), brine (50 ml) and dried over $MgSO_4$. No purification was performed. ESI-MS: m/z 289 $[M + H]^+$.

N^{α}-[*N*-(*tert*-Butyloxycarbonyl)-2-aminoethyl]phenylalanine *tert*-butyl ester (7b). Aldehyde **6** (0.5 g, 3.14 mmol) and H–Phe–O*t*Bu · HCl (**5b**; 0.68 g, 2.62 mmol) were dissolved in 3% AcOH in MeOH (6.5 ml) containing molecular sieves (3 Å). To this $NaBH_3CN$ (0.22 g, 3.53 mmol) was slowly added. After stirring for 2 h, EtOAc (50 ml) and 5% aq. $NaHCO_3$ (20 ml) were added. The aqueous layer was extracted with EtOAc (3 × 25 ml) and the combined organic layers were washed with 5% aq. $NaHCO_3$ (20 ml), brine (20 ml) and dried over $MgSO_4$. After evaporation, the residue was purified by column chromatography (3% TEA in hexane–EtOAc, 5 : 1 → 1 : 1 v/v), affording **7b** in 66% yield (0.63 g, 1.73 mmol). ^{1}H NMR ($CDCl_3$): $\delta = 7.27$ (m, 5H, Phe arom.), 4.97 (br s, 1H, NH), 3.35 (t, $J = 7.1$ Hz, 1H, αPhe), 3.15–3.08 (m, 2H, BocNCH$_2$CH_2N), 2.87 (d, $J = 7.1$ Hz, 2H, βPhe), 2.74 (m, 1H, BocNCH$_2$CH_2N), 2.56 (m, 1H, BocNCH$_2$CH_2N), 1.45 (s, 9H, Boc), 1.36 (s, 9H, *t*Bu); ESI-MS: m/z 365 $[M + H]^+$.

N^{α}-[*N*-(*tert*-Butyloxycarbonyl)-2-aminoethyl]-N^{α}-(9-fluorenylmethoxycarbonyl)alanine *tert*-butyl ester (8a). Crude **7a** was dissolved in DCM (30 ml), then DiPEA (2.19 ml, 12.6 mmol) and Fmoc–OSu (2.44 g, 7.22 mmol) were added. After stirring overnight the solvent was evaporated and the residue purified by column chromatography (hexane–EtOAc, 3 : 2 v/v), affording **8a** in 39% (from **5a**) yield (1.24 g, 2.43 mmol). ^{1}H NMR ($CDCl_3$): $\delta = 7.75$ (m, 2H, Fmoc arom.), 7.56 (m, 2H, Fmoc arom.), 7.38–7.30 (m, 4H, Fmoc arom.), 4.92 (br s, 1H, NH), 4.54–4.07 (br m, 4H, CH_2 Fmoc + CH Fmoc + αAla), 3.48–2.89 (br m, 4H, NC_2H_4N), 1.41 (br s, 21H, *t*Bu + Boc + βAla); ESI-MS: m/z 511 $[M + H]^+$, 455 $[M - t\text{Bu}]^+$.

N^{α}-[*N*-(*tert*-Butyloxycarbonyl)-2-aminoethyl]-N^{α}-(9-fluorenylmethoxycarbonyl)phenylalanine *tert*-butyl ester (8b). Compound **7b** (0.63 g, 1.73 mmol) was dissolved in DCM (9 ml), then DiPEA (0.59 ml, 3.48 mmol) and Fmoc–OSu (0.67 g, 2.0 mmol) were added at 0 °C. After stirring overnight the solvent was evaporated and the residue purified by column chromatography (hexane–EtOAc, 4 : 1 v/v). Product **8b** was obtained in 31% yield (0.32 g, 0.54 mmol). ^{1}H NMR ($CDCl_3$): $\delta = 7.78$ (m, 2H, Fmoc arom.), 7.69–7.55 (m, 2H, Fmoc arom.), 7.43 (m, 2H, Fmoc arom.), 7.34 (m, 2H, Fmoc arom.), 7.27–7.10 (m, 5H, Phe arom.), 5.12 (br s, 1H, NH), 4.98 (br m, 1H, αPhe), 4.66 (m, 1H, CH Fmoc), 4.45 (m, 1H, CH_2 Fmoc), 4.27 (m, 1H, CH_2 Fmoc), 3.28 (br d, $J = 6.1$ Hz, 2H, βPhe), 3.08 (m, 1H, NC_2H_4N), 2.87 (m, 1H, NC_2H_4N), 2.66 (m, 1H, NC_2H_4N), 2.46 (m, 1H, NC_2H_4N), 1.50 (s, 9H, Boc), 1.42 (s, 9H, *t*Bu); ESI-MS: m/z 588 $[M + H]^+$.

N^{α}-[2-Aminoethyl]-N^{α}-(9-fluorenylmethoxycarbonyl)alanine (9a). Compound **8a** (1.24 g, 2.43 mmol) was dissolved in DCM (1.6 ml) and TFA (9 ml) was added. After stirring for 2 h, the solvent was co-evaporated with toluene, resulting in a brown oil of **9a**.

N^{α}-[2-Aminoethyl]-N^{α}-(9-fluorenylmethoxycarbonyl)phenylalanine (9b). Compound **8b** (0.32 g, 0.54 mmol) was dissolved in DCM (0.34 ml) at 0 °C and TFA (2 ml) was added. After stirring for 1 h, the solvent was co-evaporated with toluene, resulting in a brown oil of **9b**.

N^{α}-[*N*-(9-Fluorenylmethoxycarbonyl)-2-aminoethyl]-N^{α}-(9-fluorenylmethoxycarbonyl)alanine (4a). The crude oil **9a** was dissolved in dioxane (30 ml), then DiPEA (1.27 ml, 7.29 mmol) and Fmoc–OSu (0.90 g, 2.67 mmol) were added and the reaction mixture was stirred overnight. EtOAc (30 ml) was added and the solution was washed with 1 M HCl (20 ml), H_2O (20 ml) and brine (20 ml), and dried over $MgSO_4$. After evaporation, the product was purified by column chromatography (1% AcOH in EtOAc–hexane, 1 : 1 v/v). The product-containing fraction was washed with 5% aq. Na_2CO_3, H_2O, brine and dried over $MgSO_4$. Product **4a** was obtained as a white powder in 58% (from **8a**) yield (0.815 g, 1.41 mmol). ^{1}H NMR (d^4 MeOD, 55 °C): $\delta = 7.78$ (m, 4H, Fmoc), 7.60 (m, 4H, Fmoc), 7.39–7.29 (m, 8H, Fmoc), 4.48–4.17 (m, 7H, CH Fmoc + CH_2 Fmoc + αAla), 3.33–2.90 (br m, 4H, NC_2H_4N), 1.37 (br m, 3H, βAla); ^{13}C NMR (d^4 MeOD, 55 °C): $\delta = 175.4$ (C_q, CO_2H), 158.6, 157.7 (C_q, 2 × C=O), 145.2 (C_q arom., 4 × Fmoc), 142.6 (C_q arom., 4 × Fmoc), 128.8, 128.1, 126.0, 120.9 (CH arom., 16 × Fmoc), 68.6 (CH_2, Fmoc), 67.5 (CH_2, Fmoc), 57.6 (CH, αAla), 56.6 (CH, 2 × Fmoc), 47.1 (CH_2, NC_2H_4N), 40.8 (CH_2, NC_2H_4N), 15.6 (CH_3, βAla); ESI-MS: m/z 575 $[M - H]^-$.

N^{α}-[*N*-(9-Fluorenylmethoxycarbonyl)-2-aminoethyl]-N^{α}-(9-fluorenylmethoxycarbonyl)phenylalanine (4b) from 8b. To crude oil **9b** in dioxane (7 ml) was added a solution of DiPEA (284 ml, 1.6 mmol) and Fmoc–OSu (202 mg, 0.60 mmol) in dioxane (2 ml). After stirring overnight, EtOAc (7 ml) was added and the solution was washed with 1 M HCl (7 ml), H_2O (7 ml) and brine (7 ml). After evaporation, the product was purified by column chromatography (EtOAc–hexane, 1 : 1 v/v), furnishing **4b** in 46% (from **8b**) yield (0.16 g, 0.25 mmol). ^{1}H NMR (d^4 MeOD, 55 °C): $\delta = 7.72$ (m, 4H, Fmoc arom.), 7.56–7.46 (br m, 4H, Fmoc arom.), 7.33 (m, 4H, Fmoc arom.), 7.24 (m, 4H, Fmoc arom.), 7.21–7.01 (m, 5H, Phe), 4.42 (br m, 2H, CH Fmoc), 4.28 (m, 2H, CH_2 Fmoc), 4.21 (m, 1H, αPhe), 4.14 (m, 2H, CH_2 Fmoc), 3.20 (br m, 2H, βPhe), 3.12–2.46 (m, 4H, NC_2H_4N); ^{13}C NMR (d^4 MeOD, 55 °C): $\delta = 175.8$ (C_q, CO_2H), 158.3 (C_q, C=O), 157.3 (C_q, C=O), 145.2, 145.2, 145.2, 145.1, 142.9, 142.6 (C_q arom., Fmoc), 139.6 (C_q arom., Phe), 129.9-123.7 (CH arom., 5 × Phe + 12 × Fmoc), 121.0 (CH arom., 4 × Fmoc), 68.5 (CH_2, Fmoc), 67.6 (CH_2, Fmoc), 64.7 (CH, αPhe), 57.1 (CH, 2 × Fmoc), 40.2 (CH_2, NC_2H_4N), 38.7 (CH_2, NC_2H_4N), 35.9 (CH_2, βPhe); ESI-MS: m/z 651 $[M - H]^-$.

N^{α}-[*N*-(*tert*-Butyloxycarbonyl)-2-aminoethyl]phenylalanine (11b). Phenylalanine **10b** (205 mg, 1.24 mmol) was suspended in MeOH (2 ml) and **6** (297 mg, 1.87 mmol) was added, followed by $NaBH_3CN$ (86 mg, 1.37 mmol). The mixture was stirred overnight, filtered and washed with MeOH, affording insoluble white solid **11b** in 52% yield (200 mg, 0.65 mmol), which was used without characterization.

N^{α}-[*N*-(*tert*-Butyloxycarbonyl)-2-aminoethyl]-N^{α}-(9-fluorenylmethoxycarbonyl)phenylalanine (12b). Bis(trimethylsilyl)acetamide (281 µl, 1.14 mmol) and DiPEA (0.11 ml, 0.65 mmol) were added to **11b** (0.20 g, 0.65 mmol) suspended in DCM (1.3 ml). The mixture was stirred until it was clear, after which Fmoc–OSu (0.23 g, 0.68 mmol) was added. After stirring overnight, the solution was co-evaporated with toluene and the residue purified by column chromatography [hexane–EtOAc (2 : 1 v/v) → AcOH–EtOAc (1 : 99 v/v)], producing **12b** in 74% yield (0.25 g, 0.48 mmol) as a colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 7.71$ (m, 2H, Fmoc), 7.52 (m, 2H, Fmoc), 7.38 (m, 2H, Fmoc), 7.36–7.07 (m, 7H, Fmoc + Phe), 4.70 (m, 1H, αPhe), 4.44 (m, 2H, CH Fmoc), 4.24 (m, 1H, CH_2 Fmoc), 4.16 (m, 1H, CH_2 Fmoc), 3.31 (m, 2H, βPhe), 3.06 (br m, 2H, BocNCH_2CH$_2$N), 2.83 (br m, 2H, BocNCH$_2$CH_2N), 1.37 (s, 9H, Boc).

N^{α}-[*N*-(9-Fluorenylmethoxycarbonyl)-2-aminoethyl]-N^{α}-(9-fluorenylmethoxycarbonyl)phenylalanine (4b) from 12b. Compound **12b** (0.24 g, 0.45 mmol) was dissolved in TFA–DCM (1 : 1 v/v, 6 ml) and stirred for 2 h. The solvent was co-evaporated with toluene and the resulting colorless oil **9b** was dissolved in dioxane (5.7 ml). DiPEA (237 µl, 1.35 mmol) and

Fmoc–OSu (0.17 g, 0.50 mmol) were added and the reaction mixture was stirred overnight. EtOAc (10 ml) was added to the mixture, which was washed with 1 M HCl (10 ml), H_2O (10 ml) and brine, dried over $MgSO_4$, filtered and evaporated. The resulting oil was purified by column chromatography [hexane–EtOAc (1 : 1 v/v) → AcOH–EtOAc (1 : 99 v/v)], furnishing **4b** as a white powder in 73% yield (0.216 g, 0.33 mmol).

***N*-[2-Aminoethyl]glycine–Gly–Gly–NH_2 dichloroplatinum (18a).** The peptide synthesis of bis-Fmoc protected tripeptide **15a** was carried out as described above starting from resin **13** (200 mg, 100 μmol). Upon cleavage of the Fmoc group in **13** the following building blocks were sequentially coupled: (1) Fmoc–Gly–OH (5 equiv.), (2) Fmoc–Gly–OH (5 equiv.), (3) **3** (5 equiv.). After the assembly was complete, the Fmoc groups in **15a** were removed and **16a** was reacted with 0.05 M K_2PtCl_4 in DMF–H_2O (9 : 1 v/v, 10 ml) for 48 h. The resin was filtered and washed with H_2O, DMF, DCM and treated with TFA–H_2O (95 : 5 v/v, 2.5 ml) for 1.5 h. After filtration, the resin was washed with TFA and the combined TFA fractions were precipitated with Et_2O. The solid was washed with Et_2O, taken up in H_2O and lyophilized. Gel permeation chromatography (HW 40, 0.01 M HCl in MeOH–H_2O, 1 : 1 v/v) afforded **18a** as a yellow powder in 58% (from **13**) yield (28.6 mg, 57.5 μmol). ^{1}H NMR (D_2O): δ = 4.06 (m, 1H, CH_2), 4.03 (AB, 2H, αGly), 3.95 (s, 2H, αGly), 3.77 (m, 1H, CH_2), 3.05–2.86 (br m, 1H, NC_2H_4N), 2.82–2.52 (br m, 3H, NC_2H_4N); ^{195}Pt NMR (D_2O): δ = −2376; ESI-MS: m/z 497 $[M + H]^+$.

***N*-[2-Aminoethyl]glycine–Gly–Arg–NH_2 dichloroplatinum AcOH (18b; mixture of diasteriomers). 18b** was prepared as described previously.[1a]

***N*-[2-Aminoethyl]alanine–Gly–Gly–NH_2 dichloroplatinum (18c; mixture of diasteriomers).** As described for **18a** with: (1) Fmoc–Gly–OH (4 equiv.), (2) Fmoc–Gly–OH (4 equiv.), (3) **4a** (4 equiv.), 0.05 M K_2PtCl_4 in DMF–H_2O (9 : 1 v/v, 12.3 ml) for 24 h. Yellow powder, 28% (from **13**) yield (14.2 mg, 27.8 μmol). ^{1}H NMR (0.1 M DCl in D_2O): δ = 4.28 (q, J = 7.0 Hz, αAla), 4.07 (m, αAla), 3.92 (AB, 2H, αGly), 3.89 (AB, 2H, αGly), 3.04 (br m, 1H, NC_2H_4N), 2.68–2.50 (br m, 3H, NC_2H_4N), 1.42 (d, J = 7.1 Hz, βAla), 1.31 (d, J = 7.0 Hz, βAla); ^{195}Pt NMR (0.1 M DCl in D_2O): δ = −2387; ESI-MS: m/z 511 $[M + H]^+$; RP HPLC: R_t = 2.25 and 2.55 min.

***N*-[2-Aminoethyl]alanine–Gly–Arg–NH_2 dichloroplatinum HCl (18d; mixture of diasteriomers).** As described for **18a** with (1) Fmoc–Arg(Pbf)–OH (4 equiv.), (2) Fmoc–Gly–OH (4 equiv.), (3) **4a** (4 equiv.), (4) 0.05 M K_2PtCl_4 in DMF–H_2O (9 : 1 v/v, 14.5 ml). The loading of bis-Fmoc protected tripeptide **15d** on the resin was 0.260 mmol g^{-1}. Yellow powder, 31% (from **13**) yield (20.2 mg, 31.2 μmol). ^{1}H NMR (0.1 M DCl in D_2O): δ = 4.21 (m, 1H, αArg), 4.21, 3.99 (2 × m, 1H, αAla), 3.88 (m, 2H, αGly), 3.10 (m, 2H, δArg), 2.90 (br m, 1H, NC_2H_4N), 2.72–2.51 (br m, 3H, NC_2H_4N), 1.78 (m, 2H, βArg), 1.71–1.42 (m, 5H, γArg + βAla); ^{195}Pt NMR (0.1 M DCl in D_2O): δ = −2371, −2386; ESI-MS: m/z 610 $[M + H]^+$; RP HPLC: R_t = 10.70 and 11.78 min.

***N*-[2-Aminoethyl]phenylalanine–Gly–Gly–NH_2 dichloroplatinum (18e; mixture of diasteriomers).** As described for **18a** with (1) Fmoc–Gly–OH (4 equiv.), (2) Fmoc–Gly–OH (4 equiv.), (3) **4b** (4 equiv.), 0.05 M K_2PtCl_4 in DMF–H_2O (9 : 1 v/v, 12.1 ml). Crude **18e** was lyophilized from 3% aq. AcOH. Yellow powder, 24% (from **13**) yield (14.3 mg, 24.3 μmol). ^{1}H NMR (0.1 M DCl in D_2O): δ = 7.24–7.09 (m, 5H, Phe), 4.15 (m, αPhe), 3.83 (m, 1H, αGly), 3.79 (s, 2H, αGly), 3.44 (AB, 1H, αGly), 3.29 (br m, 1H, NC_2H_4N), 3.03 (d, J = 8.1 Hz, 2H, βPhe), 2.68 (br m, 1H, NC_2H_4N) 2.60–2.35 (br m, 2H, NC_2H_4N); ^{195}Pt NMR (0.1 M DCl in D_2O): δ = −2403; ESI-MS: m/z 587 $[M + H]^+$; RP HPLC: R_t = 10.37 and 10.73 min.

***N*-[2-Aminoethyl]phenylalanine–Gly–Arg–NH_2 dichloroplatinum HCl (18f; mixture of diasteriomers).** As described for **18a** with: (1) Fmoc–Arg(Pbf)–OH (4 equiv.), (2) Fmoc–Gly–OH (4 equiv.), (3) **4b** (4 equiv.). The loading of bis-Fmoc protected tripeptide **15f** on the resin was 0.244 mmol g^{-1}. A part (1/2) of **16f** (0.122 g, 50 μmol) was reacted with 0.05 M K_2PtCl_4 in DMF–H_2O (9 : 1 v/v, 8 ml), affording **18f** in 14% (from **13**) yield (5.1 mg, 7.1 μmol). ^{1}H NMR (0.1 M DCl in D_2O): δ = 7.37–7.24 (m, 5H, Phe), 4.38–4.17 (m, 2H, αPhe + αArg), 3.78 (AB, 2H, αGly), 3.38–3.10 (m, 5H, βPhe + δArg + NC_2H_4N), 2.97–2.78 (br m, 1H, NC_2H_4N), 2.73–2.45 (m, 2H, NC_2H_4N), 1.85 (m, 2H, βArg), 1.64 (m, 2H, γArg); ^{195}Pt NMR (0.1 M DCl in D_2O): δ = −2368, −2383; ESI-MS: m/z 686 $[M + H]^+$; RP HPLC: R_t = 14.39 min.

Cytotoxicity studies

A2780 (human ovarian carcinoma) and A2780cisR (cisplatin-resistant) cell lines were grown as monolayers in DMEM (Gibco BRL™, Invitrogen Corporation, Netherlands) supplemented with 10% fetal calf serum (Gibco, Paisley, Scotland), penicillin (100 units ml^{-1}; Duchefa, Netherlands) and streptomycin (100 μg ml^{-1}; Duchefa, Netherlands) in a humidified 6% CO_2, 94% air atmosphere, 37 °C. Cells were passed after trypsinization when the plates were 80–90% full.

Cell growth inhibition by the platinum compounds was determined using the MTT assay.[8] After trypsinization, cells were divided into 96-wells plates at concentrations of 3–5 × 10^3 cells per well in 100 μl growth medium. The cells were incubated for 24 h prior to drug testing to allow cell adhesion. Stock solutions (1 mg ml^{-1} complete millipore) of the compounds were freshly prepared. The dilutions (5 step dilutions) were prepared in complete medium. The concentrations used were 0.2, 0.1, 0.02, 0.01, 0.002 mg ml^{-1}. Each concentration was tested in quadruplicate, using 100 μl per well added to the 100 μl of complete medium in the well. In the control group, 100 μl of complete medium was added. The plates were incubated for 72 h, after which MTT (5 mg ml^{-1}, 50 μl) was added to each well, followed by incubation for 2 h. The medium was discarded and the blue formazan crystals were dissolved in DMSO (100 μl). Optical density of the wells was measured at 590 nm using a Biorad 550 microplate reader. The IC_{50} values (drug concentration that results in 50% reduction of cell growth with respect to the untreated control) were determined graphically using GraphPad Prism® software (version 3.05, 2000).

Acknowledgements

This research was supported by the Council for Chemical Sciences of The Netherlands Organization for Scientific Research (CW–NWO) and by The Netherlands Foundation for Technical Sciences (STW). Support and sponsorship by COST Action D8/00097 (Biocoordination Chemistry) and D20/0003 (Metal Compounds in the Treatment of Cancer) is kindly acknowledged. The authors wish to thank Johnson & Matthey (Reading, UK) for their generous loan of K_2PtCl_4.

References

1 (*a*) M. S. Robillard, A. R. P. M. Valentijn, N. J. Meeuwenoord, G. A. van der Marel, J. H. van Boom and J. Reedijk, *Angew. Chem., Int. Ed.*, 2000, **39**, 3096; (*b*) S. van Zutphen, M. S. Robillard, G. A. van der Marel, H. S. Overkleeft, H. den Dulk, J. Brouwer and J. Reedijk, *Chem. Commun.*, 2003, 634; (*c*) M. S. Robillard, M.

Bacac, H. van den Elst, A. Flamigni, G. A. van der Marel, J. H. van Boom and J. Reedijk, *J. Comb. Chem.*, 2003, **5**, 821.
2 (*a*) N. Y. Sardesai, K. Zimmermann and J. K. Barton, *J. Am. Chem. Soc.*, 1994, **116**, 7502; (*b*) N. Y. Sardesai, S. C. Lin, K. Zimmermann and J. K. Barton, *Bioconjugate Chem.*, 1995, **6**, 302; (*c*) M. Lee, J. E. Simpson, Jr., A. J. Burns, S. Kupchinsky, N. Brooks, J. A. Hartley and L. R. Kelland, *Med. Chem. Res.*, 1996, **6**, 365; (*d*) R. H. Terbrueggen, T. W. Johann and J. K. Barton, *Inorg. Chem.*, 1998, **37**, 6874 and references therein; (*e*) J. W. Trauger, E. E. Baird and P. B. Dervan, *J. Am. Chem. Soc.*, 1998, **120**, 3534; (*f*) X. Huang, M. E. Pieczko and E. C. Long, *Biochemistry*, 1999, **38**, 2160; (*g*) C. A. Hastings and J. K. Barton, *Biochemistry*, 1999, **38**, 10042; (*h*) P. G. Baraldi, R. Romagnoli, A. Duatti, C. Bolzati, A. Piffanelli, N. Bianchi, C. Mischiati and R. Gambari, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1397.
3 J. Luo and T. C. Bruice, *J. Am. Chem. Soc.*, 1997, **119**, 6693.
4 M. S. Robillard, N. P. Davies, G. A. van der Marel, J. H. van Boom, J. Reedijk and V. Murray, *J. Inorg. Biochem.*, 2003, **96**, 331.
5 (*a*) K. L. Dueholm, K. H. Petersen, D. K. Jensen, M. Egholm, P. E. Nielsen and O. Buchardt, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1077; (*b*) G. Haaima, A. Lohse, O. Buchardt and P. E. Nielsen, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1939; (*c*) A. Farèse, N. Patino, R. Condom, S. Dalleu and R. Guedj, *Tetrahedron Lett.*, 1996, **37**, 1413; (*d*) A. Püschl, S. Sforza, G. Haaima, O. Dahl and P. E. Nielsen, *Tetrahedron Lett.*, 1998, **39**, 4707; (*e*) B. Falkiewicz, K. Kowalska, A. S. Kolodziejczyk, K. Wisniewski and L. Lankiewics, *Nucleosides Nucleotides*, 1999, **18**, 353; (*f*) Y. Wu and J.-C. Xu, *Tetrahedron*, 2001, **57**, 8107; (*g*) B. Falkiewicz, W. Wisniowski, A. S. Kolodziejczyk, K. Wisniewski and L. Lankiewics, *Nucleosides Nucleotides*, 2001, **20**, 1393; (*h*) B. Falkiewicz, A. S. Kolodziejczyk, B. Liberek and K. Wisniewski, *Tetrahedron*, 2001, **57**, 7909.
6 G. Bitan, D. Muller, R. Kasher, E. V. Gluhov and C. Gilon, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1501.
7 D. R. Bolin, I.-I. Sytwu, F. Humiec and J. Meienhofer, *Int. J. Peptide Protein Res.*, 1989, **33**, 353.
8 H. Tada, O. Shiho, K. Kuroshima, M. Koyama and K. Tsukamoto, *J. Immunol. Methods*, 1986, **93**, 157.