Targeting Platinum Compounds: synthesis and biological activity

Zutphen, S.van

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Combinatorial discovery of new asymmetric *cis* platinum anticancer complexes made possible using solid-phase synthetic methods*

Abstract - To efficiently access asymmetric *cis*-platinum(II) complexes for biological evaluation a new solid-phase synthesis was designed. This synthesis was used for the preparation of a small library of platinum compounds. Several compounds from this library revealed promising activity during a cytotoxicity screen. Two active compounds were therefore synthesised on a larger scale and tested more extensively against a larger panel of cell lines, confirming their high potential as antitumour compounds. The work presented illustrates how a combination of a new methodology and established techniques can speed up the search for platinum complexes with improved cytotoxic profiles compared to cisplatin.

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5.1 INTRODUCTION

Since the serendipitous discovery of its antitumour properties by Rosenberg in 1965 [1,2], cisplatin has become a well-established antineoplastic agent. Severe side effects and the occurrence of drug-resistance in cisplatin chemotherapy, however, have encouraged the search for platinum-based drugs with improved cytotoxic profiles. Over the years thousands of platinum compounds have been synthesized and tested for their anticancer activity, yet only a handful of compounds has been approved for clinical use [3,4]. The limited success in platinum anticancer research appears to be caused by the relative lack in structural diversity encountered in the reported compounds. Most cisplatin analogues described in literature contain either two identical amine ligands, a chelating didentate ligand, or an ammine and an amine ligand. Asymmetrical complexes with two different amine substituents coordinating in *cis* position present an attractive, but as yet less studied class of platinum complexes with cytotoxic potential [5-11]. An efficient synthetic method that could be applied for a combinatorial evaluation of this class of compounds would enable in-depth analysis leading to a better understanding of these complexes. As a result, novel lead compounds in the development of platinum drugs with favourable properties compared to the parent compound may be discovered.

When generating asymmetric cisplatin analogues the key intermediate is a platinum species that contains two chloride ligands in *cis* position and one amine ligand *cis* to a position that can be easily substituted by a second amine. This coordination site may be either vacant, or contain a weakly bound ligand, such as a halogen [12]. In solution it is difficult to form such a mono-amine complex: in the reaction of $[PtX_4]^{2-}$ with one equivalent of N-donating ligand there is little or no control over mono- versus disubstitution, in fact, there is even a preference to form the *cis* diamine species. The three-step procedure, as described by Rochon et al. [5]

involving a bridged dinuclear intermediate, is a useful method to access asymmetrical *cis*-diamineplatinum complexes in solution. However, this procedure is rather delicate and unsuitable for the rapid preparation of an array of asymmetric cisplatin analogues. An attractive alternative route to the key intermediate monoamine-platinum species is to immobilize an N-donating ligand onto a suitable polymeric resin of low substitution. This can be treated with $[PtX_4]^{2-}$ to yield exclusively the monosubstituted product $[PtNH_2RX_3]^-$ due to the pseudo-dilution effect. Introduction of a second amine to this resin bound platinum species followed by cleavage from the resin can thus yield the desired asymmetric cisplatin analogue. Since general advantages of solid-phase synthesis, such as easy work-up and purification of intermediates, use of excess reagents and simple synthetic protocols, would apply to these reactions, this approach is more suited for a parallel synthesis of a library of asymmetric cisplatin analogues [13,14].

The current chapter presents a solid-phase synthetic strategy for the preparation of asymmetrically substituted cisplatin analogues and its application to produce a 3 × 4 library of novel complexes. After characterisation, the crude complexes were subjected to a cytotoxicity screen, which revealed several complexes with activities comparable to cisplatin. Two of the more active compounds were selected and resynthesised on larger scale in solution using earlier described methods [5,10]. These two compounds were analysed in detail and their IC_{50} value were determined in two different cell lines, sensitive and resistant to cisplatin. The activities found confirmed the observations from the cytotoxicity screen.

5.2 *LIBRARY SYNTHESIS*

The solid-phase strategy is exemplified by the synthesis of compound **13** as follows (Scheme 5.1). Condensation of N-Fmoc-protected glycinol (**6**) obtained by $NaBH_4$ reduction of the corresponding amino acid [15,16], to trichloroacetimidate-activated Wang resin (**1**)[17], followed by piperidine-mediated deprotection afforded the immobilised primary amine **3**. Platination of this amine was effected with K_2PtCl_4 in H_2O:NMP (1:9). This solvent mixture proved to be optimal both with respect to solubility of the platinum salt and swelling of the resin. At this stage the pseudo-dilution effect and the use of excess K_2PtCl_4 ensured the exclusive formation of the resin-bound platinum mono-amine complex **4**. Treatment of **4** with a five-fold excess of methylamine (**9**) resulted in the resin-bound *cis*-platinum complex **5**. Here the greater *trans*-directing effect of Cl^- compared to the NH_2 group of glycinol dictates the exclusive formation of the *cis* isomer. Under acidic conditions the desired complex was released from the resin and precipitated with diethyl ether, to yield complex **13** as a pale yellow powder. Following the same protocol the complete 3 × 4 library was assembled using

three amino alcohols (**6**-**8**) derived from N-α-Fmoc-glycine, N-α-Fmoc-N-ε-Boc-lysine and N-α-Fmoc-O-tert-butyl-serine, respectively and four amines: methylamine (**9**), isopropylamine (**10**), dl-1-amino-2-propanol (**11**) and 2-dimethylaminoethylamine (**12**) (Figure 5.1).

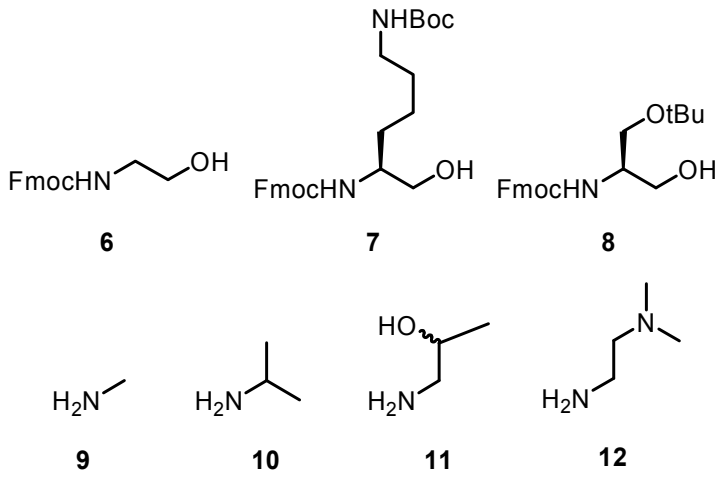
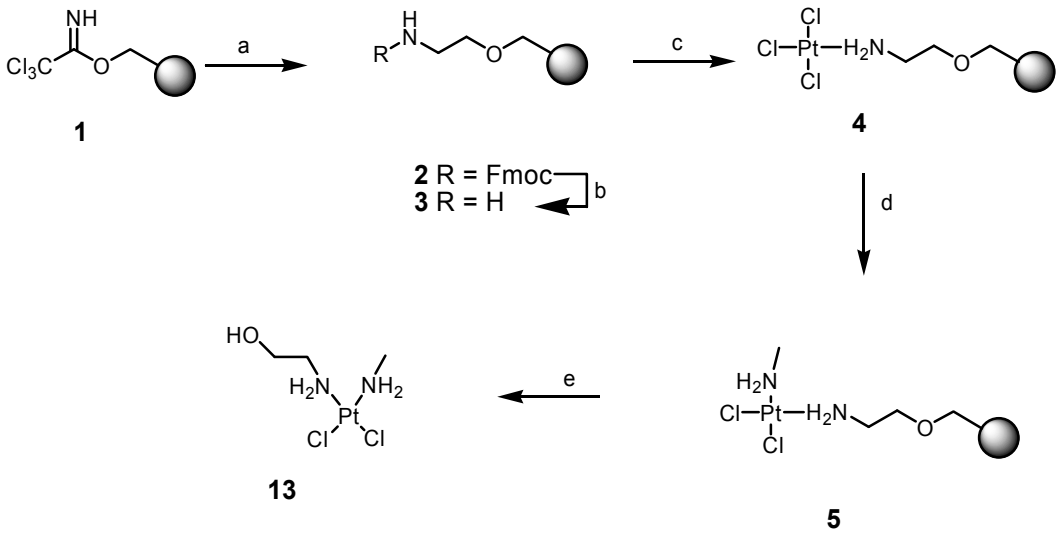


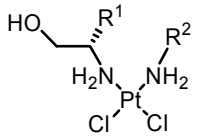
Figure 5.1: Amino alcohols (**6**-**8**) and amines (**9**-**12**) used for the library synthesis.



Scheme 5.1: Solid-phase synthesis of complex **13**. Reagents and conditions: (a) N-Fmoc glycine alcohol (**6**), BF_3•Et_2O, dry THF; (b) 20% piperidine, NMP; (c) 5 equiv K_2PtCl_4, H2O:NMP (1:9); (d) 5 equiv methylamine (**9**), NMP; (e) 90% TFA, 5% H_2O, 5% DCM cleavage.

All compounds, illustrated in scheme 5.2, were readily isolated and ^{1}H NMR and ESI-MS confirmed their structural integrity. The overall yield of the precipitated solids was found to be 18% on average, calculated by weight relative to the initial loading of amino alcohol [18]. GC analysis revealed the compounds to be of purity ranging from 50 to 95% with most compounds in the 70-80% range. Since it was intended to use the crude compounds for biological analysis, the nature of the impurities was investigated.

The ^{1}H NMR spectra suggest the presence of some unplatinated amino alcohol as an impurity. The ESI-MS spectra showed no platinum species present in the crude products, as would be easily identified by the distinctive platinum isotope pattern, other than the desired products. Therefore, it was concluded that the impurities must consist of unplatinated amino alcohol and fragments of the linker or the resin liberated under the acidic cleavage conditions. These impurities are not expected to contribute towards any cytotoxicity.



Entry	R^1	R^2
13	H	Me
14	H	
15	H	OH
16	H	N
17	NH_2	Me
18	NH_2	
19	NH_2	OH
20	NH_2	N
21	OH	Me
22	OH	
23	OH	OH
24	OH	N

Scheme 5.2: Library of asymmetric cisplatin analogues (**13**-**24**).

5.3 *CYTOTOXIC SCREENING*

In the ensuing biological evaluation, each of the 12 compounds were tested for their cytotoxicity on human ovarian carcinoma cell lines sensitive and resistant to cisplatin, A2780 and A2780R respectively. To this end, the individual compounds were added to cell cultures at three different concentrations. As a control, cisplatin was included in the assay. After 48 h the amount of viable cells remaining was determined spectrophotometrically using the MTT assay [19]. The growth inhibitory effect of the compounds was then calculated relative to untreated controls and compared to cisplatin (Table 5.1).

Table 5.1: Cytotoxic data for library compounds in the human ovarian carcinoma cell lines, showing the percentage survival relative to untreated controls of compounds 13-24 and cisplatin (cDDP) at three different concentrations.

	A2780			A2780R		
entry	5.5 μM	16.5 μM	50 μM	5.5 μM	16.5 μM	50 μM
13	75	58	39	99	77	59
14	72	49	33	98	100	78
15	82	60	41	99	98	75
16	91	77	52	84	100	98
17	91	86	63	98	93	95
18	94	82	56	97	100	89
19	85	90	78	95	97	93
20	95	90	84	100	92	90
21	89	88	63	98	96	90
22	88	82	57	100	95	91
23	74	60	39	96	90	81
24	88	84	54	99	95	96
cDDP	43	32	11	76	58	6

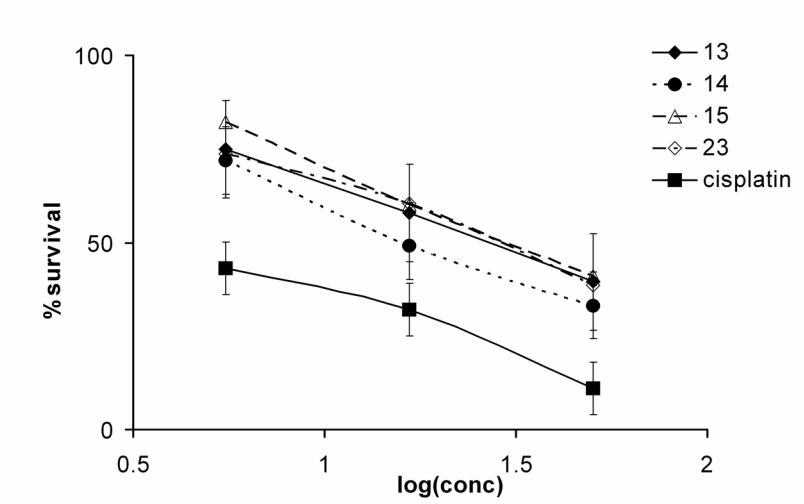


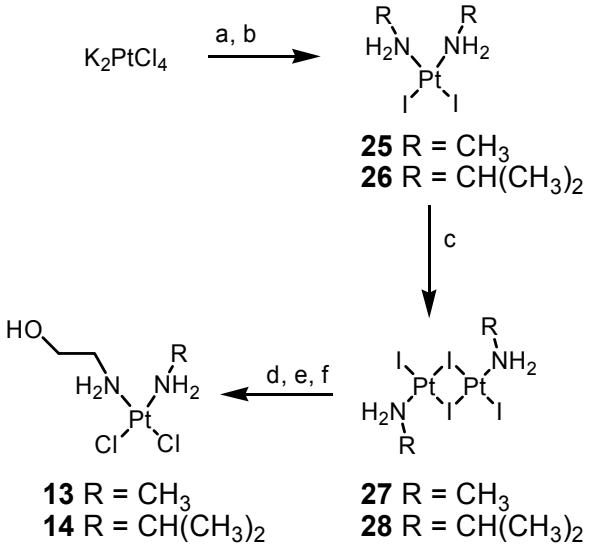
Figure 5.2: Cytotoxic profile of selected library members in A2780 human ovarian carcinoma cell line.

In the cisplatin-sensitive cell line a number of compounds show a significant inhibitory effect, not observed in the cisplatin-resistant cell line. This illustrates that the compounds do not overcome cisplatin resistance. On the other hand, although none of the compounds are as active as cisplatin, at least four compounds (**13**, **14**, **15** and **23**) from this small library exhibit promising activity at the lower concentrations in the A2780 cell line (Figure 5.2).

Remarkably, in other combinatorial evaluations of platinum antitumour drugs far fewer active compounds were discovered [20,21]; this observation further illustrates the high potential of asymmetric cisplatin analogues.

5.4 SYNTHESIS AND BIOLOGICAL ANALYSIS OF "HIT" COMPOUNDS

Encouraged by this success the synthesis of compounds **13** and **14** was carried out in larger amounts. For this purpose the solution-phase synthesis approach involving a bridged dinuclear platinum intermediate was used (Scheme 3) [5,10].



Scheme 5.3: Solution-phase synthesis of complex **13** and **14**. Reagents and conditions: (a) 10 equiv of KI in H_2O, 1.5 h; (b) 2 equiv methylamine (**9**) or isopropylamine (**10**) in H_2O, 3h; (c) 17 equiv hypochloric acid in H_2O, 7 days; (d) 2 equiv of aminoethanol in DMF, 24 h; (e) 1.9 equiv of $AgNO_3$ in DMF: H_2O (1:1), 24 h; (f) 4 equiv of KCl in DMF: H_2O (1:1), 48 h.

By addition of methylamine or isopropylamine to K_2PtCl_4 the symmetric *cis*-diaminedichloroplatinum complexes **25** and **26** were formed, respectively [22]. Treatment of these with 17 equiv of perchloric acid yielded bridged compounds **27** and **28** [23]. These reactions could be monitored by the change in colour of the solution from yellow to brown, and by following the ^{195}Pt NMR shift changing from around -3300 ppm to around -3995 ppm for both complexes. In either case the bridged complex could be degraded by the addition of 2-aminoethanol, to give the desired complexes **13** and **14** pure in an overall yield of 89% and 54%, respectively. ^{195}Pt NMR spectroscopy was used to confirm coordination of the amino-ethanol nitrogen and to prove the *cis* geometry of the complexes. The observed chemical

shifts, -2238 and -2211 ppm for **13** and **14** respectively, are typical for the *cis*[PtN_2Cl_2] chromophore. Both coordination of the amino-ethanol oxygen, or *cis* to *trans* isomerism would have lead to a significant downfield shift. The *cis* geometry of the complexes is further supported by the two sharp bands observed around 320 cm^{-1} in the IR spectra, assigned to the stretching of the two different Pt-Cl bonds present [24]. Clearly during the reaction (at pH 8) the amino-ethanol oxygen does not coordinate to platinum and also *cis* to *trans* isomerism does not occur. ^{1}H NMR and ESI-MS were used to complete the characterisation of the complexes.

As the next research objective the new batches of complexes **13** and **14** were subjected to *in-vitro* cytotoxicity assays in 4 different cell lines and compared to cisplatin. Again the A2780 and A2780R human ovarian cancer sensitive and resistant to cisplatin were used in addition to the L1210 and L1210R cell lines, mouse leukaemia sensitive and resistant to cisplatin. Growth inhibition of the complexes was measured using the MTT assay and calculated relative to blank controls. The calculated IC_{50} values, defined as the concentration of compound where 50% of the cell growth is inhibited, are given in table 5.2. These data confirm the results found during the library screen. Both compound **13** and **14** show high cytotoxic activity in the A2780 cell line, while the A2780R cell line possesses a high degree of resistance to both cisplatin and the two new compounds. In the L1210 cell line compound **14** displays particularly promising activity, whereas compound **13** shows only moderate activity. However, the resistance of the L1210R cell line is partially overcome by both new compounds, since their activity is reduced by factor 2-3 compared to a factor 15 for cisplatin. Clearly these results confirm that compounds **13** and **14** are two promising antitumour active compounds.

Table 5.2: The IC_{50} values for compounds 13 and 14 with respect to cisplatin (cDDP) in four different cell lines, A2780, A2780R human ovarian carcinoma sensitive and resistant to cisplatin and L1210, L12010R mouse leukaemia sensitive and resistant to cisplatin.

	A2780	A2780R	L1210	L1210R
compound	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
13	4.47 ± 0.30	>50	20.8 ± 5.5	35.7 ± 14.8
14	3.98 ± 0.23	34.6 ± 9.2	4.24 ± 1.0	13.8 ± 3.4
cDDP	1.43 ± 0.45	24.8 ± 8.4	1.03 ± 0.16	15.0 ± 1.5

5.5 CONCLUSION

Using the techniques presented in the current study, a relatively less-studied class of cisplatin analogues, composed of two different amine ligands and two chloride leaving groups in *cis* position is made readily accessible. Libraries of this class of asymmetric cisplatin analogues can now be prepared with relative ease through the use of solid-phase combinatorial synthesis. By screening the crude compounds obtained from the small library presented here, several new platinum compounds with cytotoxic profiles similar to cisplatin were identified. The validity of this approach is given by the synthesis and testing of two of the most active compounds from the library on a larger scale, allowing purification, in depth analysis and testing against a wider panel of cell lines. In follow-up studies the structure of these two "hit" compounds was used as a lead in the search for platinum compounds with improved activity compared to cisplatin [25].

5.6 EXPERIMENTAL SECTION

5.6.1 General

Materials were obtained from commercial suppliers and used without further purification. Trichloroacetimidate Wang resin was obtained from Novabiochem (0.77 mmol/g). 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. The water signal for the spectra taken in D_2O was minimized using a WATERGATE pulse sequence. FTIR spectra were obtained on a Perkin Elmer Paragon 1000 FTIR spectrophotometer equipped with a Golden Gate ATR device, using the diffuse reflectance technique in the range 4000-300 cm^{-1} (resolution 4 cm^{-1}). MS-spectra were taken on a ThermoFinnegan AQA ESI-MS. GC was performed on a Varian 3400 with a WCOT Fused Silica column with CP-Sil-5CB stationairy phase. A gradient of 10 ^oC/min was used between a starting temperature of 135 ^oC and final temperature of 265 ^oC.

*5.6.2 Synthesis of **6-8***

To N-Fmoc-protected amino acid (3 mmol) in dry THF (2 ml) was added N-methyl morpholine (3 mmol, 0.33 ml) followed by isobutyl chloroformate (3 mmol, 0.39 ml) at -15 ^oC under nitrogen. After 1 h the precipitate was removed by filtration and washed with a little dry THF. The combined filtrates were cooled to -15 ^oC and a solution of $NaBH_4$ (9

mmol, 342 mg) in water (3 ml) was added, followed by another 200 ml of water. Compounds **6-8** precipitated as white solids and were filtered off, washed with water followed by hexane and dried.

N-α-Fmoc glycine alcohol **6**: ^{1}H NMR ($CDCl_3$, TMS) δ (ppm): 3.33 (m, 2H; *CH_2*NH), 3.69 (m, 2H; *CH_2*OH), 4.21 (t, J = 6.1 Hz, 1H; CH, Fmoc), 4.43 (d, J = 6.5 Hz, 2H; CH_2, Fmoc), 7.26-7.42 (m, 4H; Fmoc Ar CH), 7.58 (d, J = 7.3, 2H; Fmoc Ar CH), 7.75 (d, J = 7.5 Hz, 2H; Fmoc Ar CH). ^{13}C ($CDCl_3$) δ (ppm): 43.5 (*CH_2*NH), 47.2 (Fmoc CH), 62.3 (*CH_2*OH), 66.7 (Fmoc CH_2), 119.9, 125.0, 127.0, 127.7 (Fmoc Ar), 143.8 (Fmoc Ar quat C). ESI-MS: *m/z*: 284 $[M+H]^+$, 306 $[M+Na]^+$.

N-α-Fmoc-N-ε-Boc-lysine alcohol **7**: ^{1}H NMR ($CDCl_3$, TMS) δ (ppm): 1.43-1.56 (m, 15H; tBu, (*CH_2)$_3$*CH_2NHBoc) 2.30 (broad s, 1H; OH), 3.09 (m, 2H; *CH_2*NHBoc), 3.63 (m, 2H; *CH_2*OH), 4.22 (t, J = 6.6 Hz, 1H; CH, Fmoc), 4.41 (m, 2H; CH_2, Fmoc), 5.01 (m, 1H; NH), 7.26-7.43 (m, 4H; Fmoc Ar CH), 7.59 (d, J = 7.4, 2H; Fmoc Ar CH), 7.76 (d, J = 7.5 Hz, 2H; Fmoc Ar CH). ^{13}C ($CDCl_3$) δ (ppm): 22.5, 29.7, 30.2 (*$(CH_2)_3$*CH_2NHBoc) 28.4 (tBu), 39.6 (*CH_2*NHBoc), 47.3 (Fmoc CH), 53.0 (*CH*NH), 64.7 (*CH_2*OH), 66.6 (Fmoc CH_2), 119.9, 125.0, 127.0, 127.7 (Fmoc Ar), 141.3, 143.9 (Fmoc Ar quat C). ESI-MS: *m/z*: 477 $[M+Na]^+$.

N-α-Fmoc-O-tert-butyl-serine alcohol **8**: ^{1}H NMR ($CDCl_3$, TMS) δ (ppm): 1.21 (s, 9H; tBu), 3.54 (m, 1H; *CH*NH), 3.61-3.87 (m, 4H; *CH_2*OH and *CH_2*OtBu), 4.24 (t, J = 6.7 Hz, 1H; CH, Fmoc), 4.45 (m, 2H; CH_2, Fmoc), 5.54 (m, 1H; NH), 7.25-7.44 (m, 4H; Fmoc Ar CH), 7.62 (d, J = 7.3, 2H; Fmoc Ar CH), 7.76 (d, J = 7.3 Hz, 2H; Fmoc Ar CH). ^{13}C ($CDCl_3$) δ (ppm): 27.3 (tBu), 47.2 (Fmoc CH), 51.6 (*CH*NH), 63.7 (*CH_2*OH), 64.6 (*CH_2*OtBu), 66.8 (Fmoc CH_2), 74.0 (*C*$(CH_3)_3$), 119.9, 125.0, 127.0, 127.7 (Fmoc Ar). ESI-MS: *m/z*: 370 $[M+H]^+$.

*5.6.3 Solid-phase synthesis of **13-24***

The trichloroacetimidate-activated Wang resin (200 mg, 0.154 mmol) was preswollen in DCM for 45 minutes and washed 3 times with dry THF. The N-Fmoc-protected amino alcohol (**6-8**, 0.231 mmol) in THF (1.5 ml) and a catalytic amount of $BF_3{\cdot}Et_2O$ were added, and the mixture was shaken for 2 h. MeOH (0.15 ml) was added and the reaction was allowed to proceed another 10 min. The solution was drained and the resin was washed 3 times with THF, methanol and DCM, before it was vacuum dried over KOH. Loading of the dry resin was determined using the Fmoc volumetric test. After treatment of the preswollen resin with 20% piperidine in NMP (3 × 10 min) and washing with NMP (3 × 5 min) 4 eq. of a 0.05 M solution of K_2PtCl_4 in H_2O:NMP (1:9) was added based on the Fmoc-loading test [18]. The resin was shaken overnight in the dark at room temperature. The solution was drained and the resin was washed several times sequentially with H_2O and NMP followed by NMP. Subsequently a five-fold excess of amine (**9-12**) based on the Fmoc-loading test was added to the resin in NMP (1 ml). The reaction was shaken overnight at room temperature and washed several times with NMP and DCM. The target material was cleaved from the resin by

treatment with 1 ml of 90% TFA, 5% DCM and 5% H_2O for 3 h. The solution was drained into 10 ml of diethyl ether and a precipitate formed instantly. An additional 1 ml of TFA solution was added to the resin and drained into the diethyl ether for washing of the resin. After 1 h at -20 ^oC the product was centrifuged, washed with diethyl ether (3 ×), redissolved in H_2O:ACN (1:1), filtered and lyophilised to give the products as pale yellow powders. The yields were calculated by weight of crude product relative to initial resin loading of amino alcohol.

13. ^1H NMR: (D_2O <5% d^3 ACN, TSP) δ (ppm): 2.56 (m, 3H; CH_3), 3.03 (m, 2H; NH_2CH_2), 3.99 (t, J = 5.10 Hz, 2H; CH_2OH). ESI-MS: *m/z*: 396 $[M+K]^+$. Purity 70%. Yield 18%.

14. ^1H NMR: (D_2O <5% d^3 ACN, TSP) δ (ppm): 1.53 (d, J = 6.4 Hz, 6H; $NH_2CH(CH_3)_2$), 3.03 (m, 2H; NH_2CH_2), 3.32 (m, 1H; NH_2CH), 4.03 (t, J = 5.2 Hz , 2H; CH_2OH). ESI-MS: *m/z*: 393 $[M^*+H]^+$. Purity 80%. Yield 8.5%.

15. ^1H NMR: (D_2O <5% d^3 ACN, TSP) δ (ppm): 1.63 (m, 3H; CH_3), 3.01 (m, 1H; NH_2CH_2CH), 3.26 (m, 2H; $NH_2CH_2CH_2$), 4.23 (t, J = 5.1 Hz, 2H; CH_2OH), 4.56 (m, 1H; NH_2CH_2CH), 5.07-5.36 (broad m, 2H; NH_2). ESI-MS: *m/z*: 406 $[M+H]^+$. Purity 50%. Yield 6.0%.

16. ^1H NMR: (D_2O <5% d^3 ACN, TSP) δ (ppm): 3.04-3.19 (m, 6H; CH_2CH_2OH, $NH_2CH_2CH_2N$), 3.25 (s, 6H; $(CH_3)_2$, 4.24 (m, 2H; CH_2OH). ESI-MS: *m/z*: 414 $[M+H]^+$. Purity 70%. Yield 9.7%.

17. ^1H NMR: (D_2O <5% d^3 ACN, TSP) δ (ppm): 1.67-1.90 (m, 6H; m, $CH(CH_2)_3$), 2.65 (m, 3H; NH_2CH, NH_2CH_3), 3.21 (m, 2H; CH_2NH_2), 4.26 (m, 2H; CH_2OH). ESI-MS: *m/z*: 430 $[M+H]^+$. Purity 79%. Yield 45%.

18. ^1H NMR: (D_2O <5% d^3 ACN, TSP) δ (ppm): 1.49 (m, 6H; $(CH_3)_2$) 1.69-1.86 (m, 6H; $CH(CH_2)_3$), 2.74 (m, 1H; NH_2CHCH_2), 3.18 (m, 3H; $NH_2CH(CH_3)_2$ CH_2NH_2), 4.27 (m, 2H; CH_2OH). ESI-MS: *m/z*: 458 $[M+H]^+$, 495 $[M+K]^+$. Purity 85%. Yield 45%.

19. (D_2O <5% d^3 ACN, TSP) δ (ppm): 1.35 (m, 3H; CH_3), 1.65-1.85 (m, 6H; $CH(CH_2)_3$), 2.71 (m, 1H; NH_2CHCH_2), 3.16 (m, 4H; CH_2NH_2, NH_2CH_2CH), 4.30 (m, 2H; CH_2OH). ESI-MS: *m/z*: 474 $[M+H]^+$, 495 $[M+Na]^+$. Purity 60%. Yield 34%.

20. ^1H NMR: (D_2O <5% d^3 ACN, TSP) δ (ppm): 1.65-1.85 (m, 6H; $CH(CH_2)_3$), 2.99-3.17 (m, 12H; $CH(CH_2)_3$, $(CH_2)_3CH_2NH_2$), $NH_2CH_2CH_2N(CH_3)_2$), 4.37 (m, 2H; CH_2OH). ESI-MS: *m/z*: 495 $[M+Na]^+$. Purity 60%. Yield 28%.

21. (D_2O <5% d^3 ACN, TSP) δ (ppm): 2.53 (m, 3H; NH_2CH_3), 3.05 (m, 1H; NH_2CH), 3.97 (m, 4H; CH_2OH). ESI-MS: *m/z*: 394 $[M^*+H]^+$. Purity 95%. Yield 2.2%.

22. (D_2O <5% d^3 ACN, TSP) δ (ppm): 2.50 (m, 3H; $CH(CH_3)_2$), 3.05 (m, 2H; NH_2CH), 3.97 (m, 4H; CH_2OH). ESI-MS: *m/z*: 456 $[M+K]^+$. Purity 70%. Yield 7.2%.

23. (D_2O <5% d^3 ACN, TSP) δ (ppm): 1.18 (d, J = 5.0 Hz, 3H; CH_3), 2.82 (m, 1H; NH_2CH), 2.98 (m, 2H; NH_2CH_2), 3.88 (m, 4H; CH_2OH), 4.13 (m, 1H; NH_2CH_2CH). ESI-MS: *m/z*: 471 $[M+K]^+$. Purity 80%. Yield 8.1%.

24. (D_2O <5% d^3 ACN, TSP) δ (ppm): 2.88-2.91 (m, 4H; $NH_2CH_2CH_2N(CH_3)_2$), 2.98 (s, 6H; $N(CH_3)_2$), 3.23 (m, 1H; NH_2CH), 4.15 (m, 4H; CH_2OH). ESI-MS: *m/z*: 410 $[M\text{-}Cl]^-$, 445 $[M+H]^+$, 486 $[M+K]^+$. Purity 50%. Yield 10%.
note: M* denotes the mass where all exchangeable protons are deuterium, as NMR samples where used in mass spectrometric analysis.

*5.6.4 Solution-phase synthesis of **13** and **14***

25: K_2PtCl_4 (0.5 g, 1.2 mmol) was dissolved in water (25 ml) and treated with KI (2.0 g, 12 mmol). After 1.5 h at room temperature methylamine (207 µl 40% wt in water, 2.4 mmol) was added quickly to the dark-red K_2PtI_4 solution. The reaction mixture was stirred for 3 h. The yellow precipitate was filtered off, washed with water, methanol and diethyl ether and dried in a vacuum oven at 40 °C overnight. ^1H NMR: (acetone-d_6): δ (ppm): 2.65 (m, 6H; CH_3), 4.45 (broad s, 4H; NH_2). Yield: 81%.
26: Synthesis as for **25** where methylamine is replaced by isopropylamine (204 µl, 2.4 mmol). ^1H NMR: (acetone-d_6) δ (ppm): 1.37 (d, J = 6.5 Hz, 12H; $NH_2CH(CH_3)_2$), 3.59 (sept, J = 6.65 Hz, 2H; NH_2CH), 4.39 (broad s, 4H; NH_2). Yield: 90%.
27: A suspension of **25** (0.4 g, 0.78 mmol) in water (2 ml) and ethanol (9 ml) was treated with 17 equiv of $HClO_4$ (70%) over a period of 7 days at room temperature. During the reaction the yellow precipitate turned into a red brown precipitate. The suspension was filtrated and the precipitate was washed with water and dried in a vacuum oven at 40 °C overnight. ^{195}Pt NMR (DMF) δ (ppm): -3967, -3981 ppm. Yield: 92%.
28: Synthesis as for **27** using **26** (0.44 g, 0.78 mmol) instead of **25**. ^{195}Pt NMR (DMF) δ (ppm): -3995. Yield: 89%.
13: Aminoethanol (12.5 µl, 0.21 mmol) in DMF (3 ml) was slowly added to a solution of **27** (0.1 g, 0.1 mmol) in DMF (5 ml) and stirred at room temperature. After 24 h $AgNO_3$ (65 mg, 0.39 mmol) was added and the solution was stirred in the dark. After 24 h the reaction mixture was centrifuged to remove the precipitated AgI and the yellow solution was treated with KCl (62 mg, 0.83 mmol) for 48 h. The solvent was removed by lyophilisation to yield the crude product. To remove excess salts, the crude complex was dissolved in acetone, filtered and evaporated to dryness to yield the product as a pale yellow powder (77 mg, 0.22 mmol) in 89% yield. ^{195}Pt NMR (DMF) δ (ppm): -2238. ^1H NMR (acetone-d_6) δ (ppm): 2.59 (m, 3H; CH_3), 2.84 (s, 1H; OH), 2.97 (m, 2H; NH_2CH_2), 3.91 (m, 2H; CH_2OH), 4.69 (broad m, 4H; NH_2). IR (cm^{-1}): 320, 316 (Pt-Cl stretching). ESI-MS: *m/z*: 382.95 $[M+Na]^+$.
14: Synthesis as for **13**, using **28** (100 mg, 0.01 mmol) to yield the product as a pale yellow powder in 54% yield. ^{195}Pt NMR (DMF) δ (ppm): -2211. ^1H NMR (acetone-d_6) δ (ppm): 1.41 (d, J = 6.5 Hz, 6H; $NH_2CH(CH_3)_2$), 2.98 (m, 2H; NH_2CH_2), 3.31 (m, 1H; NH_2CH), 3.93 (m, 2H; CH_2OH), 4.65 (broad m, 4H, NH_2). IR (cm^{-1}): 324, 316 (Pt-Cl stretching). ESI-MS: *m/z*: 391 $[M+Li]^+$.

5.6.5 Cytotoxicity assay and IC_{50} determination

The A2780 and A2780R were a generous gift from Dr. J.M. Perez (Universidad Autónoma de Madrid, Spain). The cells were grown as monolayers in Dulbecco's modified Eagle's Medium supplemented with 10% fetal calf serum (Gibco, Paisley, Scotland), penicillin (100 units/ml: Dufecha, Netherlands) and streptomycin (100 μg/ml: Dufecha, Netherlands). The L1210 and L1210R cell lines were cultured in McCoy's 5a medium supplemented with 10% fetal calf serum (Gibco, Paisley, Scotland), penicillin (100 units/ml: Dufecha, Netherlands) and streptomycin (100 μg/ml: Dufecha, Netherlands). During growth the cells grew partly in suspension and partly adherent to the flask.

For the cell growth assay, cells were pre-cultured in 96 multi-well plates for 48 h at 37 ^{o}C in a 7% CO_2 containing incubator and subsequently treated with 100 μl of compound at 100, 33 and 11 μmol in triplicate. After 48 h, MTT [3-(4',5'-dimethylthiazol 2'-yl)-2,5-diphenyltetrazolium bromide] in PBS (100 μl at 2.5 mg/ml) was added and the cells were incubated for 2 h. The solution was carefully removed and the remaining crystals dissolved in 100 μl of DMSO after which the absorbance at 590 nm of each well was determined using a plate reader. The growth inhibition was determined relative to untreated controls. The experiments were performed in triplicate.

For the IC_{50} determination of compounds **13**, **14** and cisplatin, cells were pre-cultured for 24 h at 37 ^{o}C in a 7% CO_2 containing incubator in 96 multi-well plates and subsequently treated with 100 μl of compound at 6 different concentrations to give final concentrations between 50-0 μmol in quadruplicate. After 72 h, MTT in PBS (50 μl at 5 mg/ml) was added and the cells were incubated for 2 h. The solution was carefully removed and the remaining crystals dissolved in 100 μl of DMSO after which the absorbance at 590 nm of each well was determined using a plate reader. The growth inhibition was determined relative to untreated controls. The experiments were performed in triplicate.

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