Mononuclear Tetraamineplatinum(II) Complexes: Synthesis, Anticancer Activity, DNA Binding, and Cellular Uptake

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Dedicated to the memory of *Luigi M. Venanzi*

The synthesis of three bis[(*tert*-butoxy)carbonyl]-protected (tetramine)dichloroplatinum complexes **2a**–**c** of formula *cis*-[$PtCl_2$(LL)] and of their cationic deprotected analogs **3a**–**c** and their evaluation with respect to *in vitro* cytotoxicity, intramolecular stability, DNA binding, and cellular uptake is reported. The synthesis comprises the complexation of $K_2[PtCl_4]$ with di-*N*-protected tetramines **1a**–**c** to give **2a**–**c** and subsequent acidolysis, yielding **3a**–**c**. The cytotoxicity of the complexes is in direct relation to the length of the polyamine. Complexes **3a**–**c** display a significant higher affinity for CT DNA as well as for cellular DNA in A2780 cells than cisplatin.

Introduction. – It is well-established that removal in cells of cisplatin- and carboplatin-induced DNA adducts contributes to tumor resistance to these drugs [1]. To overcome this problem, a plethora of platinum(II) complexes that interact with DNA in a manner distinct from that of the parent drugs have been designed and evaluated with respect to the distortion of DNA, as well as the interaction with proteins and antitumor activity [2]. The participation of polyamines in cellular proliferation and differentiation, protein synthesis, as well as DNA replication and stabilization has created much interest in the development of chemotherapeutic agents to modulate these functions [3]. Furthermore, since many mammalian cells have a polyamine uptake system, such systems are potential vehicles for delivering cytotoxic agents into cells [3c][4]. In this respect, linear polyamines like putrescine, spermidine, and spermine have been successfully employed as DNA-interacting elements coordinated to Pt-complexes [5]. However, these studies have mainly dealt with mono- or didentate dinuclear Pt-complexes, as well as tri- or tetradentate mononuclear Pt-complexes. The development of cisplatin-like, mononuclear didentate dichloro(polyamine)platinum complexes, retaining the biologically relevant linear cationic polyamine structure, have remained largely unexplored. Here we report a straightforward synthesis route towards the three cationic dichloro(tetramine)platinum complexes **3a**–**c**, varying in the length of the polyamine, *via* the selectively diprotected tetramine complexes **2a**–**c** (*Scheme*). These complexes were investigated to establish how charge, polarity, steric features, and length of the tetramine affect the *in vitro* antiproliferative activity, binding to DNA, and cellular uptake.

Experimental. – *General*. Ligands **1a** – **c** were prepared as described earlier [6]. NMR Spectra: *Bruker DPX-300* spectrometer, 5-mm multi-nucleus probe; constant temp. monitored by a variable-temp. unit; δ(H) and δ(C) in ppm rel. to $SiMe_4$ as an external reference (=0 ppm), δ(Pt) rel. to external $K_2[PtCl_4]$ (= −1614 ppm), *J* in Hz; measurements at pH 7.2 were carried out in 50 mM KD_2PO_4. Electrospray ionization (ESI) MS: in *m*/*z*.

*{Di(*tert*-butyl)* N*-{3-{[2-(Amino-*κ*N)ethyl]amino-*κ*N}propyl}*-N,N′*-(ethane-1,2-diyl)bis[carbamate]}dichloroplatinum(II)* (**2a**). To a soln. of $K_2[PtCl_4]$ (43.6 mg, 0.105 mmol) in H_2O/MeOH 3:4 (3.5 ml) ligand **1a** (37.9 mg, 0.105 mmol) was added, and the soln. was stirred overnight in the dark. H_2O was added and the mixture was left to precipitate at −20°. After centrifugation of the yellow suspension, the solid was washed with H_2O, the product taken up in EtOH and precipitated with Et_2O, and the precipitate washed with Et_2O and dried *in vacuo:* 31.8 mg (48%) of **2a**. Yellow powder. ^{1}H-NMR (CD_3CN, 50°): 1.41 (*s*, 9 H); 1.46 (*s*, 9 H); 1.82 (*m*, 1 H); 2.07 (*m*, 1 H); 2.54 (*m*, 1 H); 2.68 (*m*, 1 H); 2.81 (*m*, 1 H); 2.95 (*m*, 2 H); 3.14–3.33 (*m*, 7 H); 4.33 (br. *s*, 1 H); 4.49 (br. *s*, 1 H); 5.29 (br. *s*, 2 H). ^{13}C-NMR ($CDCl_3$): 26.3 (CH_2); 28.4 (2 *Me*$_3$C); 39.4 (CH_2); 45.0 (CH_2); 46.7 (CH_2); 47.1 (CH_2); 51.3 (CH_2); 55.5 (CH_2); 79.0 (Me_3C); 80.1 (Me_3C); 155.8 (2 CO). ^{195}Pt-NMR ($CDCl_3$): −2301. ESI-MS: 626 ($[M+H]^+$). Anal. calc. for $C_{17}H_{36}Cl_2N_4O_4Pt$: C 32.59, H 5.79, N 8.94; found: C 32.68, H 5.76, N 8.63.

*{Di(*tert*-butyl)* N*-{4-{[2-(Amino-*κ*N)ethyl]amino-*κ*N}butyl}*-N,N′*-(ethane-1,2-diyl)bis[carbamate]}dichloroplatinum(II)* (**2b**). As described for **2a**, with $K_2[PtCl_4]$ (163.1 mg, 0.393 mmol), H_2O/MeOH 3:1 (9.8 ml), and **1b** (147.0 mg, 0.393 mmol). The precipitate was filtered off, washed with H_2O, and dissolved in CH_2Cl_2 (2 ml). The soln. was filtered, the yellow filtrate treated with Et_2O, and the resulting precipitate centrifuged, washed with Et_2O (3 ×), and dried *in vacuo:* 220.0 mg (87%) of **2b**. Yellow powder. ^{1}H-NMR (CD_3CN, 55°): 1.41 (*s*, 9 H); 1.44 (*s*, 9 H); 1.56 (*m*, 3 H); 1.79 (*m*, 1 H); 2.53 (*m*, 1 H); 2.66 (*m*, 1 H); 2.81 (*m*, 1 H); 2.96 (*m*, 2 H); 3.11–3.28 (*m*, 7 H); 4.32 (br. *s*, 1 H); 4.50 (br. *s*, 1 H); 5.21 (br. *s*, 1 H); 5.27 (br. *s*, 1 H). ^{13}C-NMR ($CDCl_3$): 24.5 (CH_2); 25.2 (CH_2); 28.4 (2 *Me*$_3$C); 39.6 (CH_2); 46.4 (CH_2); 47.1 (CH_2); 53.0 (CH_2); 55.3 (CH_2); 65.8 (CH_2); 79.2 (Me_3C); 79.9 (Me_3C); 155.5 (CO); 156.0 (CO). ^{195}Pt-NMR ((D_7)DMF): −2344. ESI-MS: 663 ($[M+Na]^+$), 541 ($[M-Boc]^+$). Anal. calc. for $C_{18}H_{38}Cl_2N_4O_4Pt$: C 33.75, H 5.98, N 8.75; found: C 33.55, H 5.36, N 9.10.

*{Di(*tert*-butyl)* N*-{6-{[2-(Amino-*κ*N)ethyl]amino-*κ*N}hexyl}*-N,N′*-(ethane-1,2-diyl)bis[carbamate]}dichloroplatinum(II)* (**2c**). As described for **2a**, with $K_2[PtCl_4]$ (132.5 mg, 0.319 mmol), H_2O/MeOH 3:4 (9.8 ml), and **1c** (128.5 mg, 0.319 mmol). The precipitate was filtered off, washed with H_2O, and dried in the air. After addition of CH_2Cl_2 (2 ml) and filtration of the resulting soln., the yellow filtrate was precipitated with Et_2O at −20°, centrifuged, washed with Et_2O (3 ×), and dried *in vacuo:* 173.6 mg (82%) of **2c**. Yellow powder. ^{1}H-NMR (10% $CDCl_3$/CD_3CN, 55°): 1.31 (*m*, 4 H); 1.41 (*s*, 9 H); 1.44 (*s*, 9 H); 1.47–1.64 (*m*, 3 H); 1.79 (*m*, 1 H); 2.54 (*m*, 1 H); 2.67 (*m*, 1 H); 2.81 (*m*, 1 H); 2.93 (*m*, 2 H); 3.12–3.27 (*m*, 7 H); 4.28 (br. *s*, 1 H); 4.49 (br. *s*, 1 H); 5.13 (br. *s*, 1 H); 5.22 (br. *s*, 1 H). ^{13}C-NMR ($CDCl_3$): 26.1 (CH_2); 26.2 (CH_2); 27.0 (CH_2); 27.8 (CH_2); 28.4 (2 *Me*$_3$C); 39.5 (CH_2); 46.1 (CH_2); 47.2 (CH_2); 53.0 (CH_2); 55.3 (CH_2); 65.8 (CH_2); 79.0 (Me_3C); 79.6 (Me_3C); 155.3 (CO); 156.1 (CO). ^{195}Pt-NMR ($CDCl_3$): −2268. ESI-MS: 707 ($[M+K]^+$), 691 ($[M+Na]^+$), 568 ($[M-Boc]^+$). Anal. calc. for $C_{20}H_{42}Cl_2N_4O_4Pt$: C 35.93, H 6.33, N 8.38; found: C 35.81, H 6.28, N 8.07.

*{*N*-[2-(Amino-*κ*N)ethyl]-*N′*-(2-aminoethyl)propane-1,3-diamine-*κ*N}dichloroplatinum(II) Dihydrochloride* (**3a**). Complex **2a** (103.6 mg, 0.165 mmol) was suspended in 0.12M aq. HCl/MeOH 9:1 (10 ml) and stirred at 50° overnight, upon which a homogeneous light yellow soln. was formed, which was evaporated. H_2O (5 ml) was added to the residue, the resulting suspension filtered, and the filtrate lyophilized. After precipitation from 0.12M aq. HCl (1 ml) with EtOH (5 ml), the resulting residue was washed with EtOH and Et_2O and dried *in vacuo:* 47.5 mg (56%) of **3a**. Yellow powder. ^{1}H-NMR (D_2O): 2.14 (*m*, 1 H); 2.44 (*m*, 1 H); 2.51–2.74 (*m*, 4 H); 2.87 (*m*, 1 H); 3.04 (*m*, 1 H); 3.25 (*m*, 2 H); 3.40 (*m*, 4 H). ^{13}C-NMR (D_2O): 24.7 (CH_2); 36.1 (CH_2); 44.9 (CH_2); 46.2 (CH_2); 47.5 (CH_2); 50.0 (CH_2); 56.5 (CH_2). ^{195}Pt-NMR (D_2O): −2403. ESI-MS: 354 ($[M-2Cl-H]^+$). Anal. calc. for $C_7H_{22}Cl_4N_4Pt$: C 16.84, H 4.44, Cl 28.41, N 11.22; found: C 16.48, H 4.20, Cl 27.88, N 10.74.

*{*N*-[2-(Amino-*κ*N)ethyl]-*N′*-(2-aminoethyl)butane-1,4-diamine-*κ*N}dichloroplatinum(II) Dihydrochloride* (**3b**). As described for **3a**, with **2b** (220.0 mg, 0.343 mmol) and 0.12M aq. HCl/MeOH 9:1 (10 ml): 139.0 mg (79%) of **3b**. Yellow powder. ^{1}H-NMR (D_2O): 1.79 (*m*, 3 H); 2.04 (*m*, 1 H); 2.54–2.76 (*m*, 4 H); 2.87–3.01 (*m*, 2 H); 3.17 (*t*, *J* = 7.43, 2 H); 3.41 (*m*, 4 H). ^{13}C-NMR (D_2O): 23.7 (CH_2); 24.7 (CH_2); 36.3 (CH_2); 44.9 (CH_2); 47.6 (CH_2); 48.5 (CH_2); 52.7 (CH_2); 56.4 (CH_2). ^{195}Pt-NMR (D_2O): −2394. ESI-MS: 441 ($[M+H]^+$). Anal. calc. for $C_8H_{24}Cl_4N_4Pt$: C 18.72, H 4.71, Cl 27.63, N 10.92; found: C 18.25, H 4.49, Cl 28.36, N 10.47.

*{*N*-[2-(Amino-*κ*N)ethyl]-*N′*-(2-aminoethyl)hexane-1,6-diamine-*κ*N}dichloroplatinum(II) Dihydrochloride* (**3c**). As described for **3a**, with **2c** (173.6 mg, 0.260 mmol) and 0.12M aq. HCl/MeOH 9:1 (10 ml): 113.0 mg

(82%) of **3c**. Yellow powder. ^{1}H-NMR (D_2O): 1.40 (*m*, 4 H); 1.69 (*m*, 3 H); 1.88 (*m*, 1 H); 2.58 (*m*, 2 H); 2.69 (*m*, 2 H); 2.91 (*m*, 2 H); 3.11 (*t*, $J = 7.70$, 2 H); 3.39 (*m*, 4 H). ^{13}C-NMR (D_2O): 25.8 (CH_2); 26.0 (2 CH_2); 27.1 (CH_2); 36.2 (CH_2); 44.8 (CH_2); 47.7 (CH_2); 48.9 (CH_2); 53.5 (CH_2); 56.2 (CH_2). ^{195}Pt-NMR ($CDCl_3$): −2390. ESI-MS: 469 ($[M+H]^+$). Anal. calc. for $C_{10}H_{28}Cl_4N_4Pt$: C 22.19, H 5.21, Cl 26.20, N 10.35; found: C 21.67, H 4.98, Cl 26.62, N 9.96.

pH Measurements. They were carried out with a *Radiometer PHM-80* pH meter by means of a *Hamilton* combination glass electrode. The pH meter was calibrated with *Fischer*-certified buffer solns. of pH 4.00, 7.00, and 11.00. The pH was corrected for the deuterium isotope effect by adding 0.4 units to the display readout.

Flameless Atomic Absorption Spectroscopy. Pt-Concentrations were determined by graphite oven flameless atomic absorption spectroscopy (FAAS) [7]. Measurements were carried out with a *Perkin-Elmer 3100-AAS* apparatus, equipped with a Pt hollow cathode lamp and an *AS-60* graphite oven autosampler. For each determination, 20 µl of sample was injected. The furnace program was: drying 120°/90 s, ashing 1300°/60 s, 20°/15 s, atomization and measurement 2650°/5 s, purging 2600°/5 s. The furnace was purged with Ar gas.

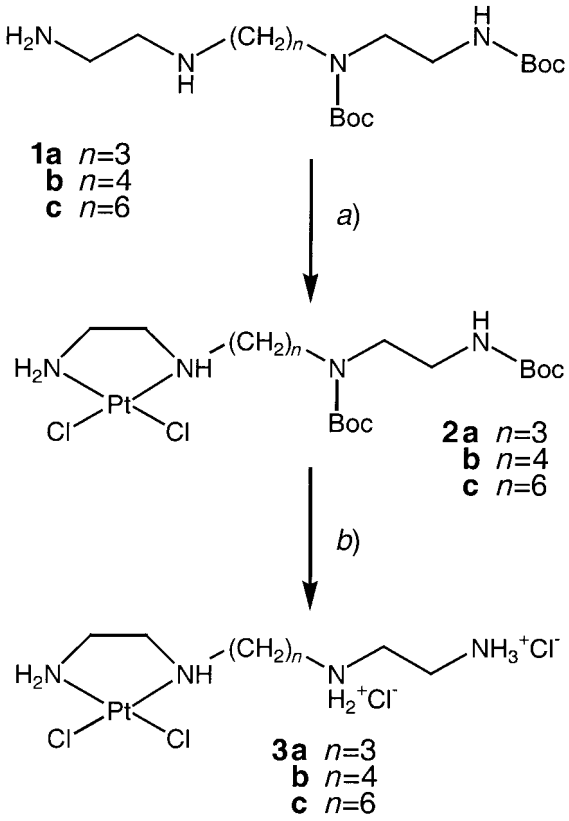
In vitro *Cytotoxicity Studies. In vitro* cytotoxicity assays were performed at the *Dr. Daniel den Hoed Kliniek* (*Rotterdam Cancer Institute*), Department of Medical Oncology (Rotterdam, The Netherlands). The seven well-characterized human tumor cell lines used were MCF 7 and EVSA-T (breast cancer), WIDR (colon cancer), IGROV (ovarian cancer), M19 MEL (melanoma), A498 (renal cancer), and H226 (non-small-cell lung cancer). The MCF 7 cell line is estrogen receptor (ER)+/progesterone receptor (PgR)+ and the cell line EVSA-T is (ER)−/(PgR)−. The cell lines WIDR, M19 MEL, A498, IGROV, and H226 belong to the currently used anticancer screening panel of the *National Cancer Institute*, USA [8]. After 2 days of preincubation of the tumor cells at 37°, the cells were exposed to the compounds for 5 days. ID_{50} Values were determined with the microculture sulforhodamine B test (SRB) [9].

r_b *Measurements.* A 1 *OD* (optical density) unit of calf thymus DNA (phenol extracted; *Amersham*, *Pharmacia Biotech*) in 1 × TE buffer (10 mM *Tris*, 1 mM Na_2H_2edta, pH 7.2) was treated with various volumes of a 100 µM stock soln. of the Pt-complexes **3a**–**c** and cisplatin, dissolved in 1 × TE buffer immediately prior to use, to achieve a final drug concentration in a volume of 100 µl. Samples were incubated for 1 h at 37°. After the incubation, the unreacted platinum and salt were removed by a *Sephadex G50* spin column. Platinated DNA soln. was collected by spinning 1.5 min at 1000 *G*. The column was washed with H_2O (100 µl) by spinning for 1.5 min at 1000 *G*. The fractions were combined and diluted to a total volume of 1 ml. DNA Concentration was determined by UV spectroscopy. The quantitation of Pt–DNA binding ($r_b = [\text{compound}]_{molar}/DNA_{bp}$ = drug molecules/base pair) was calculated on the basis of DNA concentration (molar extinction coefficient $\varepsilon_{260} = 16800\ cm^{-1}\,M^{-1}$ per base pair), and the amount of Pt was measured by FAAS.

Intracellular DNA Binding in A2780 Ovarian Cancer Cells. A2780 Human ovarium cells were a gift from Dr. *J. M. Pérez*, Universidad Autónoma de Madrid, Spain. The cell lines were grown as monolayers in *Dulbecco*'s modified *Eagle*'s Medium (DMEM) supplemented with 10% fetal-calf serum (*Gibco*, Paisley, Scotland), penicillin (100 units/ml; *Duchefa*, Netherlands), and streptomycin (100 µg/ml; *Duchefa*, Netherlands) in a humidified 6% CO_2, 94% air atmosphere. The cells were passed after trypsinization. For the uptake/DNA-binding experiment, cells were grown in P6 plates with 2 ml of medium/well, until a concentration of *ca.* $1 \cdot 10^7$ cells. At this point, the medium was replaced by 2 ml of serum-free medium. Fresh stock solns. of the Pt-complexes were added to a final concentration of 200 µM. Each experiment was performed in duplicate. After 1 h of incubation (37°, 6% CO_2, dark), medium was removed and the cells were washed twice with 1 ml of PBS (0.1M phosphate buffer in 0.15M NaCl). Then, the cells were trypsinized and centrifuged at 1000 *G* for 10 min. At this point, the cell pellets may be stored at −20° for future use. DNA was isolated from the cells by the following procedure. Cell pellets were suspended in 300 µl of buffer (10 mM *Tris*, pH 8, 150 mM NaCl, 10 mM Na_2H_2edta). Then, proteinase K soln. (5 µl) was added and mixed thoroughly with the cells. Finally, 10% SDS (50 µl) was added, and the resulting mixture was heated at 50–55° for 1.5 h. The solns. were transferred to 2-ml *Eppendorf* phaselock tubes, and extracted with $CHCl_3$/phenol/pentan-1-ol 24:25:1 (300 µl), and subsequently with $CHCl_3$/pentan-1-ol 24:1 (300 µl). The DNA was precipitated by adding ^{i}PrOH (600 µl) to the H_2O layer. The DNA pellet was washed once with cold 70% EtOH, and then dissolved in *milli-Q* H_2O (1 ml). The DNA concentration was determined by measuring the UV absorption at 260 nm. From this absorption, the concentration of base pairs was calculated by means of an average molar extinction coefficient per base pair of $\varepsilon_{260} = 16800\ M^{-1}\,cm^{-1}$. The Pt-concentration was measured by FAAS. From the Pt-concentration, the actual drug concentration was derived. Combining FAAS and UV results, the drug molecules per base pair ratio (r_b) was calculated.

Results. – *Synthesis.* The known [6] bis[(*tert*-butoxy)carbonyl]-protected tetramine ligands **1a**–**c** were reacted with $K_2[PtCl_4]$ in H_2O/MeOH to yield complexes **2a**–**c** as yellow precipitates in 48, 87, and 82% yield, respectively (*Scheme*). Acidolysis of **2a**–**c** in 0.12M aq. HCl/MeOH afforded the hydrochloride salts **3a**–**c** (56, 79, and 82% yield, resp.). The identity and purity of the complexes were fully confirmed by NMR spectroscopy (^1H, ^{13}C, ^{195}Pt), ESI-MS, and elemental analysis.

Scheme



a) **2a**: $K_2[PtCl_4]$, H_2O/MeOH 3:4, r.t., 1 d, 48%; **2b**: $K_2[PtCl_4]$, H_2O/MeOH 3:1, r.t., 1 d, 87%; **2c**: $K_2[PtCl_4]$, H_2O/MeOH 3:4, r.t., 1 d, 82%. *b*) **3a**–**c**: 0.12M aq. HCl/MeOH 9:1, 50°, 1 d; **3a** 56%; **3b** 79%; **3c** 82%.

In vitro *Cytotoxicity Studies.* Cytotoxicity was tested in a panel of seven human tumor cell lines: H226 (non-small-cell lung cancer), A498 (renal cancer), M19 (melanoma), IGROV (ovarian cancer), WIDR (colon cancer), and EVSA-T and MCF 7 (breast cancer) (*Table*). The IC_{50} values of **2a**–**c** are high compared to cisplatin. However, these values decrease with increasing spacer length, resulting in the highest cytotoxicity for **2c** and the lowest for **2a**. The deprotected tetramine complexes **3a**–**c** display slightly lower IC_{50} values compared to their bis-protected analogs **2a**–**c**. The sequence of activity of **3a**–**c** is the same as for **2a**–**c**, *i.e.*, the complexes appear to be more active as the spacer length increases.

Table. IC_{50} *Values* [μM] *Determined in Seven Human Tumor Cell Lines by the Microculture Sulforhodamine B* (SRB) *test*

	MCF 7	EVSA-T	WIDR	IGROV	M19	A498	H226
2a	> 90	> 90	> 90	> 89.7	> 90	> 90	> 90
b	> 90	62.9	71.1	46.9	> 90	> 90	> 90
c	47.6	41.6	41.5	39.5	42.3	49.7	63.4
3a	> 125	> 125	> 125	> 125	> 125	> 125	> 125
b	111	> 115	35.9	48.5	120.5	> 115	> 115
c	27.1	> 115	11.3	7.4	43.7	33.4	27.5
cisplatin	2.3	1.4	3.2	0.6	1.9	7.5	10.9

Intramolecular Stability of Complexes **3a**–**c** *at pH 7.2.* To establish whether or not the linear cationic complexes **3a**–**c** retain their linear structure at physiological pH, the complexes were dissolved in D_2O, yielding solutions with a pH ranging from 3.4–4.6, which were submitted to ^{195}Pt-NMR spectroscopy: the original signals were recorded at −2404, −2396, and −2396 ppm, resp., corresponding to a PtN_2Cl_2 chromophore [10]. Subsequently, the pH was raised to 7.2 with phosphate buffer, and the δ(Pt) were followed for 1 h. For compounds **3b** and **3c**, δ(Pt) remained unchanged, whereas for **3a**, δ(Pt) was −3149, corresponding to a N_4 environment for platinum(II) [10]. These results indicate that at pH 7.2, the appended ethanediamine moiety of **3a** can displace the 2 Cl-ligands to give a tetradentate mononuclear Pt-complex. The increased linker length of the appended ethanediamine group seems to prevent this intramolecular displacement in **3b** and **3c** under these conditions.

Calf Thymus DNA Binding. To investigate the effect of the spacer length of complexes **3a**–**c** on DNA binding and especially to assess if **3a** retains its linear structure, the reactivity of these complexes with calf thymus (CT) DNA was studied. The complexes and cisplatin were incubated with CT DNA for 1 h at various concentrations ranging from 10 to 80 μM at 37° and pH 7.2. Subsequently, the DNA concentration was measured by UV, and the Pt content was determined with FAAS. From this information, the r_b values (the number of drug molecules bound per base pair) were calculated, and these numbers are depicted in *Fig. 1*. From the results, it is evident that complexes **3b** and **3c** display a higher DNA-binding activity than **3a**. However, **3a** still exhibits a significant higher affinity for DNA than cisplatin, which suggests that **3a** is present in its reactive linear form.

Binding to Cellular DNA in A2780. The cellular uptake and subsequent binding to DNA of the (polyamine)platinum complexes **3a**–**c** and cisplatin were studied with A2780 ovarian cancer cells. After incubation of A2780 cells for 1 h at equimolar (200 μM) concentrations of the platinum compounds, DNA was isolated, and the r_b value was determined by UV and FAAS. The r_b values are depicted in *Fig. 2*. From *Fig. 2* it is clear that compounds **3a**–**c** again bind more efficiently to DNA in the A2780 cells than cisplatin. Although **3a**–**c** display a similar DNA binding efficiency, a small trend seems to be present, with, surprisingly, **3a** now showing the highest DNA binding and **3c** the lowest.

Discussion and Conclusions. – The (tetramine)platinum compounds **2a**–**c** and **3a**–**c** were successfully synthesized from the diprotected tetramine ligands **1a**–**c**. Although

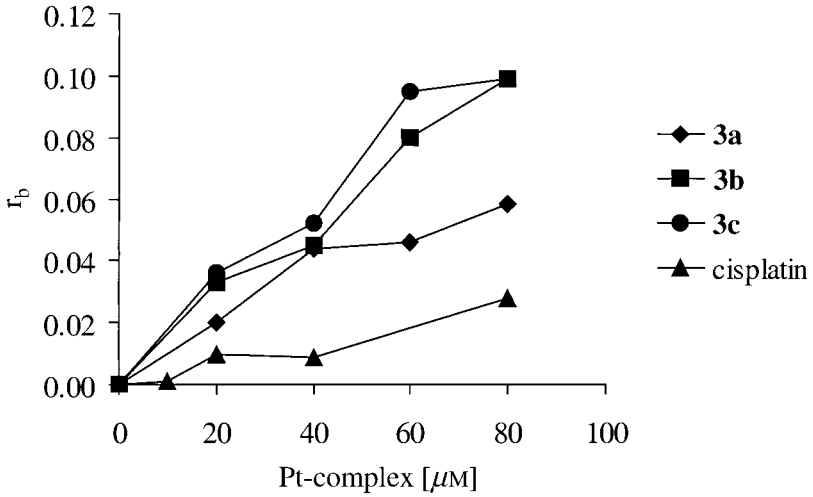


Fig. 1. *Quantitation of Pt-DNA binding* (r_b) vs. *dose* [μM] *of platinum complexes* **3a**–**c** *and cisplatin, under similar conditions*

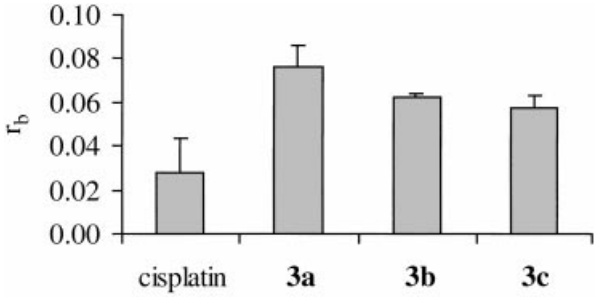


Fig. 2. *Molecules bound per base pair* (r_b) *to DNA isolated from A2780 cells after incubation with 200* μM *of platinum complexes* **3a**–**c** *and cisplatin under similar conditions*

it has been shown [2a] that some Pt-complexes bearing a side chain functionalized with a [(*tert*-butoxy)carbonyl]- (Boc-)protected amine, or other apolar groups, exhibit low IC_{50} values, complexes **2a**–**c** were found not to be very active. The two Boc groups might sterically inhibit DNA-adduct formation and membrane passage. However, the cytotoxicity increases as the linker length increases. The increasing lipophilicity might enhance membrane transport and counter the steric effect of the Boc groups. This correlation could also imply that the increasing linker length decreases the steric hindrance of the Boc groups upon DNA platination. Previously, it has also been shown that Pt-compounds with a (Boc-amino)-functionalized side chain often are more cytotoxic than their deprotected and protonated counterparts [2a]. To evaluate the effect of the Boc groups and the linear cationic polyamine character on anticancer activity, the Boc groups were removed, resulting in **3a**–**c**. The sequence of *in vitro* cytotoxic activity of **3a**–**c** was found similar to that of **2a**–**c**, leading to the

consideration that, in the present case, the cytotoxicity is mainly dependent on the length of the polyamine and less on the presence or absence of the Boc groups. Although it was shown by ^{195}Pt-NMR that **3a** undergoes an intramolecular displacement of the Cl-ligands at pH 7.2 to give the unreactive N_4-tetradentate complex, this phenomenon does not seem to occur in the buffers used for the DNA binding and cellular uptake experiments. Moreover, in the cellular DNA-binding experiment, **3a** shows the highest binding. The medium used in this study contains a physiological chloride concentration and might suppress hydrolysis of the Cl-ligands and subsequent intramolecular coordination of the appended ethanediamine moiety. From this, it is reasonable to assume that the inactivity of **3a** in the *in vitro* anticancer studies is not due to the rearrangement to the unreactive N_4-tetradentate complex, but originates from the linear structure and is comparable to the inactivity of **2a**. The DNA-binding experiments demonstrate that the biologically relevant polyamine structures of **3a**–**c** result in a greater affinity for DNA than cisplatin, and possibly, a better cellular uptake as well. However, these experiments do not clarify the increasing cytotoxicity with increasing length of the polyamines **2a**–**c** and **3a**–**c**. The origin of this sequence of activity may not principally involve a difference in cellular uptake or DNA affinity and could also lie in the molecular structures of the adducts of the (polyamine)platinum complexes with DNA. This aspect is currently under investigation and will be reported in due course.

This research has been sponsored by the *Netherlands Foundation for Technical Sciences* (*STW*, project 349-4427) and the *Swiss National Science Foundation*. The authors are indebted to the EU for a grant as Host Institute in the EU Program *Human Capital and Mobility*. Sponsorship by *COST* (Action D8/00097/97; Biocoordination Chemistry) is kindly acknowledged. The authors wish to thank *Pharmachemie BV* for the cytotoxicity tests and *Johnson & Matthey* (Reading, UK) for their generous gift of $K_2[PtCl_4]$.

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Received June 5, 2001