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Monoamineplatinum(II) complexes conjugated to water-soluble carrier polymers for chemotherapeutic applications

Carol W N Mbonyana, Eberhard W Neuse and Axel G Perlwitz
Department of Chemistry, University of the Witwatersrand, WITS 2050, Republic of South Africa

In the light of the observed carcinostatic activity of the monoamineplatinum complexes $K[PtCl_3(NH_3)]$ and $K[PtCl_3$(t-butylamine)], it has been of interest in this laboratory to develop water-soluble, antineoplastic conjugates in which square-planar platinum complex structures of the monoamineplatinum type are linked to suitable carrier polymers possessing water solubility for improved pharmacokinetics. In the present paper the synthesis is described of conjugates in which each platinum atom is coordinated to a single, primary amine ligand provided by a water-soluble polyaspartamide-type carrier. Microanalytical data suggest the remaining three coordination sites on the metal center to be occupied on average by one aquo and two chloro ligands. The carriers, prepared by a known method from polysuccinimide by stepwise aminolytic ring-opening, are designed so as to feature randomly placed hydrosolubilizing units and metal-binding units in a ratio of 3:1, thus providing spatial insulation between the latter and minimize intramolecular interaction between the platinum complexes incorporated subsequently. Platination of the carriers is brought about by treatment with K_2PtCl_4 in aqueous solution at 25–60 °C in the pH range 5–6, and the polymer–platinum conjugates are purified and isolated in 50–70% yield by aqueous-phase dialysis and freeze-drying. The extent of platination attained depends *inter alia* on the Pt/NH_2 feed ratio (equivalents of tetrachloroplatinate per carrier base unit); in optimal cases complete metal coordination to the carrier-attached primary-amine ligands is achieved with a feed ratio of 1.4:1. The conjugates, initially showing complete solubility in water, tend to undergo an ageing process on storage believed to involve intermolecular solid-state interaction of the bound platinum complexes with proximate amine sites, resulting in gradual loss of solubility. In frozen aqueous solution, however, the conjugates are stable for extended periods of time.

Keywords: Platinum, polymer, amine, carcinostatic, polymers, polyaspartamide, complexes

INTRODUCTION

Metal coordination compounds derived from both Main Group elements and transition metals have in recent years increasingly attracted the attention of biomedical and pharmaceutical researchers on account of numerous reports of bioactivity, notably antineoplastic effectiveness, and several coordination compounds of platinum have reached the stage of specialized clinical application as anticancer drugs. For recent reviews, see, for example, Refs 1–4. Severe toxic side effects, however, are a hallmark of most heavy-metal compounds, and of the platinum complexes in particular, representing a major obstacle to routine administration. Moreover, most organometallic and coordination compounds of potential therapeutic utility, including complexes of titanium, rhodium, ruthenium, iron, tin and copper, are poorly soluble or altogether insoluble in aqueous media and thus fail to lend themselves to simple and uncomplicated medical application by intravenous or intraperitoneal techniques. One of the most promising approaches adopted in present-day pharmacological research towards overcoming these deficiencies utilizes the reversible binding (anchoring) of toxic, insoluble and/or excessively labile drug species to suitably constructed macromolecular carrier molecules. Drug–polymer conjugation of this kind offers a number of potential pharmacological advantages, which include: (1) ease and simplicity of intravenous or intraperitoneal administration and distribution of the drug in the body's central circulation system with minimal depletion by hydrolytic, catabolic and other elimination mechanisms; (2) facilitated cell entry

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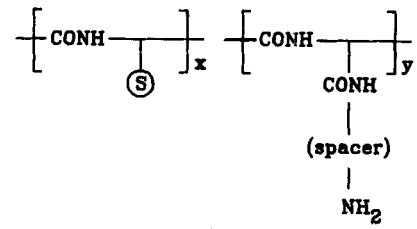
through pinocytosis (a mechanism predominantly geared for macromolecular entrants), thus minimizing problems caused by drug polarity and resistance; and (3) delayed drug release with resultant control of drug serum levels and concomitantly reduced toxicity, brought about by proper drug attachment and judicious choice of the factors determining release kinetics. The overall pharmacokinetic benefits arising from polymer–drug conjugation may be reflected in a significant enhancement of the drug's therapeutic index, a requirement of paramount importance for anticancer agents. The topic has been reviewed by various authors,[5–7] most recently by Hoes and Feijen.[8]

As part of a comprehensive programme directed towards the development of water-soluble macromolecular platinum drugs possessing enhanced therapeutic effectiveness in the treatment of cancerous diseases, we previously described the synthesis of polymer-anchored *cis*-dichloroplatinum–ethylenediamine coordination compounds.[9] In that type of drug-anchoring system the metal is firmly bound to the carrier through a chelating diamine ligand representing a biofissionable side-chain component of the carrier polymer. The present project aims at the development of polymeric platinum complexes in which the metal is bound to a single amine ligand attached to the carrier, with three metal-binding sites occupied by chloride and water ligands. We were prompted to investigate conjugates of this kind by the reported (and rather unexpected) anticancer activity of the monoamineplatinum salts $K[PtCl_3(NH_3)]$ and $K[PtCl_3(TBA)]$ (TBA = *t*-butylamine).[10,11] A representative synthetic approach to a series of water-soluble polymeric monoamineplatinum(II) complexes is described in this paper.

RESULTS AND DISCUSSION

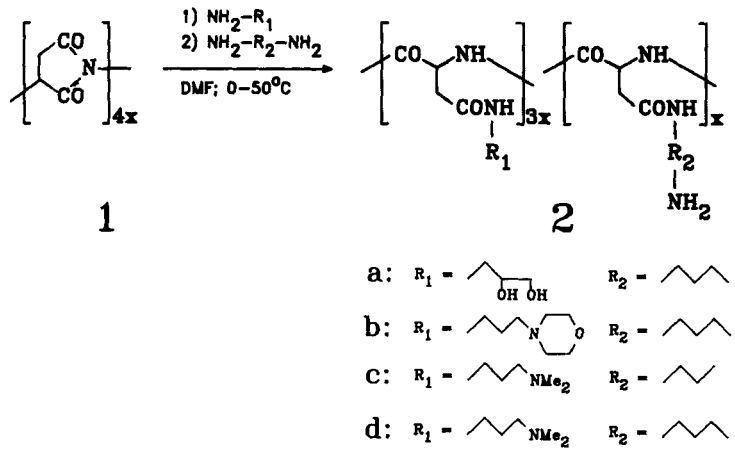
Carrier synthesis

The carrier polymers chosen, of the general structure shown below, resemble the carriers developed in previous studies, in that the two types of repeat unit comprise α,β-D,L-aspartamide segments randomly placed along the polymeric main chain. The first unit, as before,[9] contains a group labelled S comprising a tertiary amine or carbinol structure. The group S serves to enhance water



solubility while, at the same time, insulating the drug-carrying units (and, hence, the ultimately attached platinum complexes) from each other so as to reduce anchoring-site density in the molecule and prevent multifunctional drug binding. In addition, in those structures in which S is represented by a tertiary-amine function with potentially cationic character, it may exert a very moderate targeting effect towards neoplastic tissue; many types of cancer cell are known to possess excess negative surface charges, causing preferential attraction of cationic drug species. Furthermore, polycations are cytotoxic agents in their own right, and for selected representatives tumoristatic properties have been observed.[12] Other structural prerequisites for appropriately functioning carrier molecules, incorporated into the basic carrier structure shown above, include (1) a highly flexible backbone (free rotation of intrachain single bonds) for increased entropy of solution; (2) amide groups as chain constituents amenable to biodegradation for ease of catabolic elimination of chain fragments following drug release; (3) presence of functional groups (e.g. $-NH_2$), capable of forming reversible bonds, as efficacious binding sites for drug attachment and release, these groups to be located at the terminals of short, non-immunogenic side segments serving as spacers between the main chain and the drug.

The specific polymer structures selected as water-soluble carriers in this work, in conformance with the aforementioned prerequisites, were copolyaspartamides of the type **2** (Scheme 1). The potential of polyaspartamides as drug-carrier polymers was addressed earlier by Drobnik *et al.*[13] Structures **2b–2d** are known,[14] and the previously described procedures, comprising the two-step aminolysis of poly-D,L-succinimide **1** (Scheme 1; α forms shown only; random placement of units), were utilized for their preparation, experimental conditions being chosen such as to restrict reaction of the diamine nucleophile to only one of the two NH_2 terminals. Polyamide **2a**, not previously described, was pre-

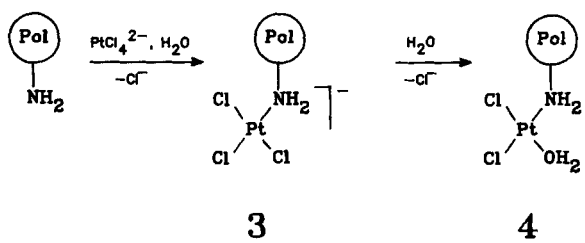


Scheme 1

pared in an analogous fashion from **1** and the two amine reactants, 1-aminopropane-2,3-diol (0.25 equivalents per base mole of substrate polymer) and excess 1,3-diaminopropane, in *N,N*-dimethylformamide (DMF) solution. It was purified and isolated by precipitation, exhaustive aqueous-phase dialysis (nominal molecular mass cut-off limit 12 000–14 000), and freeze-drying. The composition of **2a** was ascertained from the relative intensities of the proton resonances in the NMR spectra, as in the previous work.[14] The ratio $R_1/R_2 = 3$ was chosen and held constant throughout, after preceding screening experiments with $R_1/R_2 = 1.5$–1.0 had revealed a strong tendency to crosslink formation in subsequent platination work.

Carrier platination

Platination reactions were performed by treatment of the carriers (Scheme 2) in aqueous solution with potassium tetrachloroplatinate(II) under conditions similar to those routinely used for non-polymeric *cis*-diamination of Pt. The Pt/NH_2 feed ratios (i.e. equivalents of K_2PtCl_4) ranged from 1.0 to 1.5. In order to suppress acid-catalysed hydrolytic degradation, the pH was

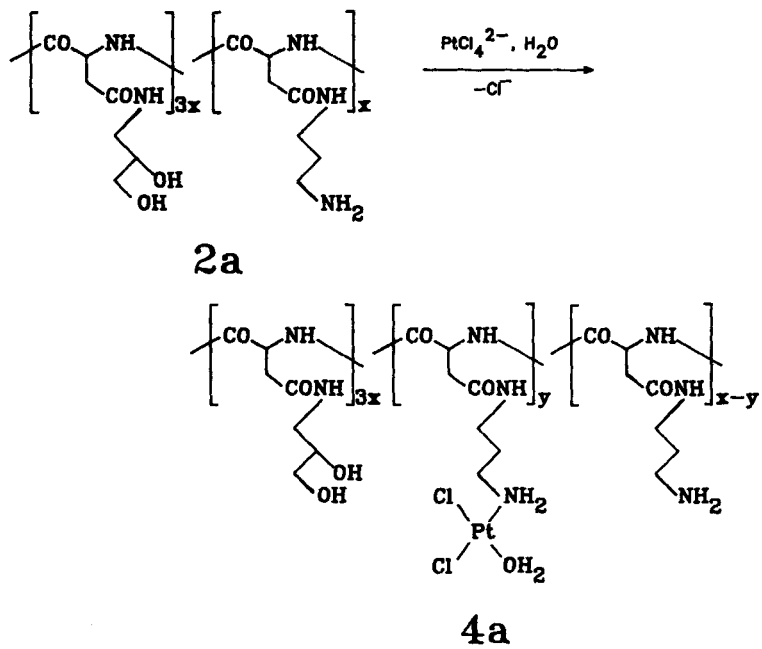


Scheme 2

maintained substantially in the range 5–6 found previously to facilitate P–N coordination with minimal metal hydroxylation and an acceptably low extent of chain fission in the aqueous medium. At sufficiently low overall reactant concentrations (0.04–0.05 mol l^{-1}), and with the primary amino groups of the carriers adequately distanced from each other to prevent inter- or intra-molecular diamination of the metal, we expected these reactions in the primary step to lead to polymeric aminetrichloroplatinum(II) salts of the type **3** and possibly, by concurrent or subsequent partial hydrolysis, to the more stable aquo–*cis*-dichloroplatinum species exemplified by **4** (Scheme 2).

The substrate polymer used in the first series of experiments was **2a**, selected here on account of its inability to undergo complicating protonation at the solubilizing sites (—OH versus $—NH_2$ in **2b–2d**). Treatment, in duplicate experiments, of **2a** in aqueous 0.04 mol l^{-1} solution* with 1.5 equivalents of K_2PtCl_4 for 22 h at room temperature and another 6 h at 60 °C with intermittent pH adjustment, followed by aqueous-phase dialysis (60 h) at pH 5 in membrane tubing possessing a 12 000–14 000 molecular-mass cut-off, pH readjustment to 3, and isolation by freeze-drying of the retentate, gave a main fraction of platinated polymeric product in an averaged yield of 52%. A second polymer fraction of lower molecular mass was collected in 20–30% yield in each one of the repeat runs by redialysis of the outer dialysis phase in tubing with a 3500 molecular-mass cut-

* Throughout this paper, polymer amounts or concentrations refer to the base mole, i.e. mole of recurring unit. For the purpose of this paper, the recurring units of both carriers and conjugates are defined by structures **2** and **4** where $x = 1$.



Scheme 3

off. Microanalyses, including platinum and chlorine determinations, performed on the main fractions of the target polymers showed these to conform essentially to structure **4a** ($y=x$) (Scheme 3) corresponding to an average of 100% platination of primary amino groups and an average of one aquo and two chloro ligands on the metal. The results are summarized in Table 1. Not unexpectedly in view of the sensitivity of the metal centre to environmental (notably pH) conditions, individual platinum and chlorine percentages showed some run-to-run scattering. The

Table 1 Conjugates **4** by tetrachloroplatinate-mediated platination of **2**[a]

Carrier polymer	Molar feed ratio[b] Pt/NH_2	Conjugate **4** Designation	Yield[c] (%)	η_{inh}[d] (ml g^{-1})	C/N[e] Found[f]	C/N[e] Calcd	Cl (%) Found[f]	Cl (%) Calcd	Pt (%) Found[f]	Pt (%) Calcd	Degree of platination[g] (%)
2a	1.0	**4a** ($y=0.6x$)	55	7	3.2	3.1	4.8	4.7	12.7	12.9	60
	1.5	**4a** ($y=x$)	52	7	3.2	3.1	7.1	6.9_5	19.0	19.1	100
2b	1.0	**4b** ($y=0.45x$)	62	10	3.5	3.3	7.8	3.1	8.6	8.6	45
	1.5	**4b** ($y=0.8x$)	68	16	3.4	3.3	9.9	5.0_5	13.7	13.9	80
2c	1.0	**4c** ($y=0.55x$)	57	12	2.8	2.7_5	9.5	4.3	11.6	11.8	55
	1.5	***4c*** ($y=x$)	53	9	3.0	2.7_5	12.1	6.8	19.0	18.8	100
2d	1.0	**4d** ($y=0.5x$)	58	11	3.0	2.8	9.2	3.9	10.9	10.7	50
	1.1	**4d** ($y=0.6x$)	53	10	2.9	2.8	9.3	4.5	12.3	12.5	60
	1.2	**4d** ($y=0.8x$)	70	20	2.8	2.8	11.8	5.7	16.0	15.7	80
	1.3	**4d** ($y=0.95x$)	62	16	3.0	2.8	12.2	6.5	17.5	17.8	95
	1.4	**4d** ($y=x$)	70	18	2.9	2.8	11.8	6.7	18.2	18.5	100
	1.5	**4d** ($y=x$)	65	17	3.0	2.8	12.0	6.7	18.9	18.5[h]	100[h]

[a] In water; 0.04–0.05 (base) mol l^{-1} in carrier polymer. [b] Equivalents of K_2PtCl_4 per carrier base unit. [c] First (main) fraction. In addition 20–30% of second fraction. [d] At 30.00 ± 0.05 °C, in water ($c=2$ mg ml^{-1}). [e] Carbon/nitrogen atomic ratio. [f] Run-to-run average. [g] Rounded off to nearest 5%. [h] 24.1% (corresponding to 130% platination) in similar experiment with dialysis period reduced to 30 h.

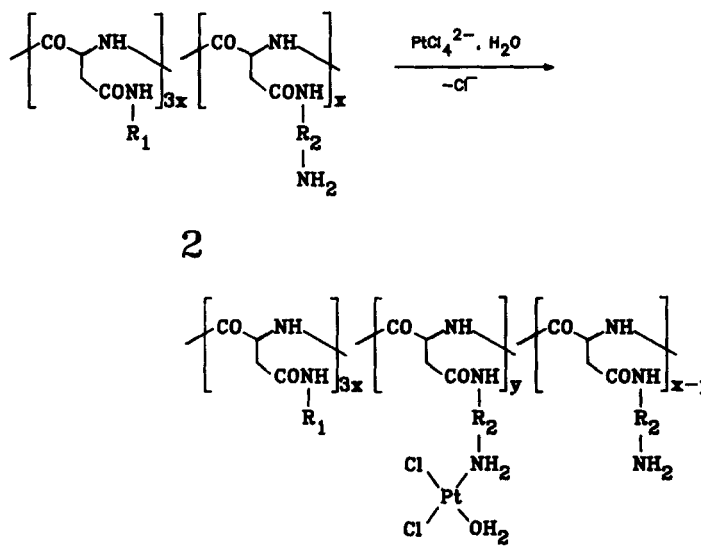
tabulated analytical data for all entries represent mean values obtained from two parallel runs for each set of variables.

Analogous experiments performed with only one equivalent of platinum salt (Table 1) gave platination products approximating **4a** ($y = 0.6x$), in which thus, on average, 60% of available polymeric amine ligands were platinum-coordinated and nearly two coordination sites of the metal were occupied by chloro ligands.

Attempts in these experiments to enforce retention of all three chloro ligands on the metal were unsuccessful. While less aquation appeared to result from conducting the runs (including the dialysis step) at lower pH (typically 3–4), this reduced main-fraction yields to less than 40%, indicating the occurrence of hydrolytic chain fragmentation. When the ultimate aqueous product solutions were acidified to pH2 just prior to freeze-drying, the chlorine contents at best increased by 1% overall, still indicating structures more in agreement with **4** than with **3** (Scheme 2); moreover, there was some *N*-protonation at residual, uncoordinated primary amino groups under these conditions, as evidenced by the downfield shift by 0.2 ppm of the broad β-CH_2 proton NMR signal, which in the spectra of the essentially non-protonated conjugates emerges at 1.9 ppm.

In the next series of experiments, performed in duplicate or triplicate, the carriers **2b** and **2c** comprising tertiary amine groups in the solubilizing units were platinated as before, Pt/NH_2 feed ratios being 1.0 and 1.5. The initial concentration was held constant at 0.05 mol l^{-1}. Yields of the water-soluble main fractions ranged from about 50 to 70%, and inherent viscosities were 8–19 ml g^{-1}. Again, second crops of polymer (20–30%) were collected in all experiments after redialysis in 3500 molecular-mass cut-off tubing. Pertinent data for the main fractions are in Table 1.

For the product polymers derived from **2b**, microanalytical results were in reasonable accord with structures **4b** ($y = 0.45x$) and **4b** ($y = 0.8x$), and **2c** gave rise to product polymers **4c** ($y = 0.55x$) and **4c** ($y = x$) (Scheme 4). Complicating the structural assignments of these macromolecules, some *N*-protonation at the tertiary amine terminals of the R_1 unit (Scheme 1) must be expected at the pH range maintained during work-up. In order to assess the degree of such protonation, several blank experiments were



Scheme 4

conducted in which the metal-free carriers **2b** and **2d** (Scheme 1), dissolved in water, were recovered from their solutions by freeze-drying under pH conditions identical with those of the platination runs, hydrochloric acid being used as before for pH adjustment. The found chlorine contents, in the vicinity of 5%, suggest about 50% of the tertiary-amine functions in the R_1 groups (more basic than any primary amine left unplatinated and therefore more susceptible to proton capture) to be protonated by the acid. Assuming this percentage range to hold as well for the platinated target compounds, one concludes on the basis of overall chlorine contents that the conjugates on average contained the platinum complex part as a monoaquo-dichloro-platinumamine species as proposed for **4a** and represented in Scheme 4. Again, however, run-to-run variations in both platinum and chlorine contents were significant (see the Experimental section below), and the polymer designations in text and tables represent averages with respect to the degree of polymer platination and platinum aquation. The minor extent of *N*-protonation at the R_1 groups has been disregarded in the simplified structures as drawn.

In the last series of platination runs, executed in duplicate or triplicate, carrier **2d** (Scheme 1) was treated with tetrachloroplatinate as before. The molar Pt/NH_2 feed ratios were 1.0, 1.1, 1.2, 1.3, 1.4 and 1.5 (Table 1). The water-soluble polymeric platinum complexes, obtained in yields of 50–70% and possessing inherent viscosities in the range of 10–20 ml g^{-1}, on average corresponded in elemental composition to the respective structures **4d** ($y=0.5x$), **4d** ($y=0.6x$), **4d** ($y=0.8x$), **4d** ($y=0.95x$), **4d** ($y=x$), and again, **4d** ($y=x$) (Scheme 4 and Table 1). The tabulated data of the last two lines indicate identical results, i.e. 100% platination of available primary-amino groups, for the two Pt/NH_2 feed ratios of 1.4 and 1.5. Clearly, a ratio of 1.4 sufficed with this polymer type for complete platinum coordination, and the additional excess of $PtCl_4^{2-}$ anion employed in the bottom-line experiments did not result in further permanent metal incorporation. It is of interest to note, however, that a separate experiment performed with a feed ratio of 1.5, yet with the dialysis period reduced to 30 h (a condition quite adequate for complete diffusion of non-polymeric platinum salt into the outer dialysis phase), gave a conjugate with a platinum content as high as 24%, corresponding to 130% platination. The excess of metal incorporated in this case was evidently bound through coordinating tertiary-amine functions and, in accordance with the rather weak nature of tertiary-amine–platinum bonding, underwent elimination as the tertiary-amine coordination was reversed on further extended dialysis in the aqueous medium. The tabulated data also show that the various carrier compositions differ slightly in their capacity for providing access of the platinating agent to the amine ligand. However, in the light of the small number of experiments and the observed run-to-run scattering of analytical data, one cannot at this stage correlate carrier side-group structures with the extent of platination achieved.

The IR spectra of the target polymers **4** proved virtually identical with those of the carrier precursors, the only difference being the emergence of a weak band near 320 cm^{-1} due to ν(Pt–Cl). No signal appeared at 1040 cm^{-1}, where hydroxo-bridged dimer species would be expected to absorb. Equally unsuitable for diagnostic purposes were the ^1H NMR spectra, which displayed the resonance patterns of the weakly protonated precursors, most indicative being the broad β-CH_2 proton signal of the aminopropyl side group with a maximum at 2.0–1.9 ppm. (For fully *N*-protonated carriers this band appears at about 2.2–2.1 ppm.) The α-CH_2 proton signal, expected to have undergone an even larger downfield shift, could not be monitored because of superposition with other methylene resonances in the 3.1–2.8 ppm region. ^{195}Pt NMR spectra should be instructive in the discrimination between $PtNCl_3$ and derived aquated species.[15,16] However, attempts to obtain useful platinum spectra have until now met with failure because of excessive line broadening caused, *inter alia*, by restricted molecular motion in these polymeric materials.

Although the freshly prepared conjugates **4** possessed complete solubility in water and thus fulfilled the target requirement of this investigation, the polymers tended to lose this important asset gradually on room-temperature storage in the solid state. This was notably the case with **4b**, **4c**, and **4d**. Apparently, as observed before,[9] slow solid-state interaction involving haloplatinum groups and tertiary-amine side-group terminals (to a minute extent perhaps also with the rather unreactive amide NH functions) on neighbouring chains gives rise to the formation of crosslinks with densities high enough to reduce and ultimately eliminate the solubility in water. While it was found to be possible to regenerate water-soluble material from the aged samples by lengthy digestion at 30–40 °C with aqueous 1 mol l^{-1} hydro-

chloric acid, thereby breaking up most of the newly formed Pt–N bonds constituting the cross-links, the recovery yields of the redialysed solutes were generally quite low (<30%) because of concomitant main-chain fragmentation. In addition, compositional changes resulted from loss of (hydrolytically cleaved) side groups. Storing the frozen solutions of the freshly prepared types **4** (0.1–0.5 mol l^{-1}) at temperatures below −10 °C represents a simpler and more convenient method of retaining water solubility. Solutions so treated have been found (upon thawing) to remain unchanged after more than six months of cold storage.

The polymeric monoamineplatinum(II) complexes will be submitted for *in vitro* screening of antineoplastic activity.

EXPERIMENTAL

General procedures

Solid-state spectra (KBr pellets) were scanned over the range 4000–200 cm^{-1}. ^1H NMR spectra (200 MHz) were recorded on D_2O solutions unless stated otherwise; chemical shifts, δ ppm, are given relative to internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate (internal tetramethylsilane with $CDCl_3$ solutions). Cannon–Fenske viscometer tubes were used for the determination of inherent viscosities, η_{inh} [in ml g^{-1}; 30.00 ± 0.05 °C; $c = 0.2$ g (100 ml)$^{-1}$]. Dialysis was performed against several batches of stationary, deionized and nitrogen-saturated water with the aid of membrane tubing of the types Spectra/Por 3 and Spectra/Por 4 (Spectrum Industries, Los Angeles, CA, USA) possessing nominal molecular-mass cut-off limits of 3500 and 12 000–14 000, respectively. Freeze-drying operations were carried out with a Virtis freeze-drier, model Bench Top 3 (−40 °C, 0.1 torr). Platinum-free freeze-dried material was routinely post-dried in a Sartorius Thermo Control Infrared Drying System (heating programme, 2 × 4 min at 75 °C). Analytical (including platinum-containing) samples were additionally dried (two days at 75 °C, 30 torr) in an Abderhalden apparatus. Under these drying conditions some 2–4% of moisture was generally retained by the hydrophilic polymers of this study; yet more forceful drying environments were not applied so as to preserve structural integrity. Platinum determinations (in duplicate or triplicate, data-averaged) were made by atomic absorption spectroscopy on sample solutions in 0.2 mol l^{-1} HNO_3, aged by storage for 24 h at room temperature prior to injection. Carbon, hydrogen, nitrogen and chlorine analyses (in duplicate, data-averaged) were performed by Robertson Laboratory, inc., Madison, NJ, USA, and by Galbraith Laboratories, Inc., Knoxville, TN, USA. In the tabulations, the carbon and nitrogen contents were expressed in terms of C/N atomic ratios, preferred here in view of the uncertainty introduced by the variable residual moisture contents of the samples. Large variations (±10% of determined values) were encountered with the chlorine analyses, which are known to be erratic in platinum-containing compounds, and the reported results should be accepted with reservation.

Reagents, reactants and solvents

Deionized water was used for all preparative work. Ethylenediamine, propylenediamine, 1-aminopropane-2,3-diol, 3-(dimethylamino)-propylamine, and 4-(3-aminopropyl)morpholine were used as received (Fluka AG, Aldrich Chemie GmbH); older portions of the amines were predried over molecular sieves 4A and were redistilled under reduced pressure in a gentle stream of nitrogen. The same treatment was used to purify the commercial *N*,*N*-dimethylformamide (DMF). Potassium tetrachloroplatinate(II) was obtained from Johnson Matthey Ltd on a loan basis. Poly-D,L-succinimide (**1**)[17,18] was prepared as described;[14] the batch selected for this work had an inherent viscosity, η_{inh} (DMF), of 0.55 ml g^{-1}, corresponding[18] to a mass-average molecular mass of approximately 58 000.

In the following text, amounts of polymeric reactants are given as base moles.

Polymeric carriers

Polyaspartamides **2b**, **2c** and **2d** were prepared by the known general procedure[14] from polysuccinimide **1** and the reactant pairs, 4-(3-aminopropyl)morpholine and propylenediamine, 3-(dimethylamino)propylamine and ethylenediamine, and 3-(dimethylamino)propylamine and propylenediamine, respectively, in DMF solution. The work-up was the same as described[14] except that the dialysis period in Spectra/Por 4 tubing was extended to five days. ^1H NMR spec-

tra were in accord with the respective compositions; η_{inh} (H_2O), 19 ml g^{-1} (**2b**), 8 ml g^{-1} (**2c**), and 12 ml g^{-1} (**2d**). The synthesis of **2a**, not previously reported, followed the same basic procedure. Briefly, 1-aminopropane-2,3-diol (7.5 mmol) was added to the nitrogen-saturated solution of **1** (10 mmol) in anhydrous DMF (20 ml) and dissolved with stirring. After 20 h at 0 °C, propylenediamine (5 mmol) was added with stirring at room temperature. The solution was left for another 20 h at 0 °C, followed by stirring for 4 h at ambient temperature. The polymer was precipitated with excess EtOH–Et_2O (1:3), dialysed in aqueous solution for three days in Spectra/Por 4 tubing, and collected from the retentate by freeze-drying. The solid product polymer, obtained in 51% yield, was not completely soluble in water, forming a turbid solution with small amounts of fine particles left suspended. The filtered solution was redialysed (48 h) in Spectra/Por 4 tubing and freeze-dried. This gave completely soluble material; η_{inh} (H_2O), 17 ml g^{-1}. Analysis Found: C, 43.36; H, 6.55; N, 15.68%; C/N 3.22. Calcd for $(C_{28}H_{49}N_9O_{14})_n$ (**2a**): C, 45.71; H, 6.71; N, 17.13%; C/N, 3.11. As the polymer tended to turn partially insoluble on extended storage, freshly prepared material was used for the platination work.

Platination experiments

All experiments were performed in duplicate or triplicate. The tabulated yield, viscosity, and microanalytical data are mean values obtained from the two or three identically conducted runs for each set of variables.

Conjugates 4a

In the experiment described below, the molar Pt/NH_2 feed ratio was 1.5. The solution of polyaspartamide **2a** (184 mg; 0.25 mmol), in water (6 ml) was saturated with nitrogen. After the addition of K_2PtCl_4 (156 mg; 0.375 mmol), the reddish solution was stirred in the stoppered flask, protected from light, for 22 h at ambient temperature and for another 6 h at 60 °C. The pH, initially 8–9, was brought down to 7 with 5 mol l^{-1} hydrochloric acid. After several hours, the pH dropped spontaneously to 5–6 and was adjusted to 6–7 with potassium carbonate; subsequently, it was maintained at 5–6 by repeated upwards adjustment, counteracting spontaneous acidification. After completion of the heating period, the solution was cooled and was dialysed for 60 h in Spectra/Por 4 tubing against several stationary batches of water acidified to pH 5 with hydrochloric acid. The filtered retentate solution, upon further acidification to pH 3 (hydrochloric acid), was freeze-dried, giving a tan-coloured conjugate (128 mg; 50.2%) as a water-soluble solid; η_{inh} (H_2O), 8 ml g^{-1}. Analysis: Found (Cl, Pt data for repeat run in parentheses): C, 30.11; H, 5.51; Cl, 7.69 (6.44); N, 11.36; Pt, 18.59% (19.41%); C/N, 3.09. Calcd for $(C_{28}H_{51}Cl_2N_9O_{15}Pt)_n$ [**4a** ($y=x$)]: C, 32.98; H, 5.04; Cl, 6.95; N, 12.36; Pt, 19.13%; C/N, 3.11.

From the combined outer aqueous phases of the Spectra/Por 4 dialysis, volume reduction by rotating evaporation and redialysis (24 h) in Spectra/Por 3 tubing gave a second fraction of water-soluble conjugate (55 mg; 21.6%); η_{inh} (H_2O), 4 ml g^{-1}.

In other experiments conducted as described above but with 0.25 mmol of K_2PtCl_4 (Pt/NH_2 feed ratio 1.0), the yield of cream-coloured, water-soluble conjugate was 56.5%; η_{inh} (H_2O), 6 ml g^{-1}. Analysis: Found: C, 33.34; H, 5.61; Cl, 5.32 (4.34); N, 12.31; Pt, 13.49% (11.85%); C/N, 3.16. Calcd for $(C_{28}H_{50.2}Cl_{1.2}N_9O_{14.6}Pt_{0.6})_n$ [**4a** ($y=0.6x$)]: C, 37.11; H, 5.58; Cl, 4.69; N, 13.91; Pt, 12.92%; C/N, 3.11.

Conjugates 4b

In the following experiment, the Pt/NH_2 feed ratio was 1.5. To the aqueous nitrogen-saturated solution (5 ml) of carrier **2b** (224 mg; 0.25 mmol) was aded K_2PtCl_4 (156 mg; 0.375 mmol), and the reddish solution was stirred in the dark for 24 h at room temperature and for 8 h at 60 °C. The pH, initially adjusted to 7 (hydrochloric acid), slowly dropped to 5–6 and was maintained at that level for the remaining reaction period. Work-up by dialysis and freeze-drying as before gave conjugate **4b** as a light-brownish, water-soluble solid in a yield of 185 mg (65.9%); η_{inh} (H_2O), 14 ml g^{-1}. Analysis: Found (Cl, Pt data for repeat run in parentheses): C, 38.79; H, 6.88; Cl, 8.98 (10.86); N, 13.03; Pt, 14.87% (12.55%); C/N, 3.47. Calcd for $(C_{40}H_{71.6}Cl_{1.6}N_{12}O_{11.8}Pt_{0.8})_n$ [**4b** ($y=0.8x$)]; C, 42.81; H, 6.43; Cl, 5.05; N, 14.98; Pt, 13.91%; C/N, 3.33.

Analogous experiments, performed at a Pt/NH_2 feed ratio of unity (0.25 mmol of K_2PtCl_4) under otherwise unchanged conditions, afforded 154 mg (60.3%) of tan-brown, water-soluble conjugate; η_{inh} (H_2O), 11 ml g^{-1}. Analysis: Found: C,

41.76; H, 7.31; Cl, 8.62 (7.01); N, 14.01; Pt, 7.41% (9.84%); C/N, 3.48. Calcd for $(C_{40}H_{70.9}Cl_{0.9}N_{12}O_{11.45}Pt_{0.45})_n$ [**4b** ($y=0.45x$)]; C, 46.97; H, 6.99; Cl, 3.12; N, 16.43; Pt, 8.58%; C/N, 3.33.

Conjugates 4c

The Pt/NH_2 feed ratio in this experiment was 1.5. Carrier **2c** (189 mg; 0.25 mmol), and K_2PtCl_4 (156 mg; 0.375 mmol), in water (5 ml) was saturated with nitrogen. The solution was stirred for 20 h at 25 °C and for another 8 h at 60 °C, turning to a blackish-brown colour during this period. conventional work-up by dialysis and freeze-drying gave 130 mg (51.5%) of tan-brown water-soluble conjugate; η_{inh} (H_2O), 10 ml g^{-1}. Analysis: Found (Cl, Pt data of two repeat runs in parentheses): C, 35.21; H, 6.72; Cl, 12.95 (12.11, 11.86); N, 13.89; Pt, 18.91% (17.85%, 20.32%); C/N, 2.96. Calcd for $(C_{33}H_{64}Cl_2N_{12}O_9Pt)_n$ [**4c** ($y=x$)]: C, 38.15; H, 6.21; Cl, 6.82; N, 16.18; Pt, 18.78%; C/N, 2.75.

A Pt/NH_2 feed ratio of 1.0 under otherwise unchanged conditions, used in analogous experiments, gave water-soluble conjugate in an average yield of 57%; η_{inh} (H_2O), 13 ml g^{-1}. Analysis: Found: C, 39.69; H, 7.40; Cl, 8.79 (10.78, 8.99); N, 16.07; Pt, 10.46% (13.03%, 11.21%); C/N, 2.88. Calcd. for $(C_{33}H_{63.1}Cl_{1.1}N_{12}O_{8.55}Pt_{0.55})_n$ [**4c**($y=0.55x$)]: C, 43.50; H, 6.98; Cl, 4.28; N, 18.45; Pt, 11.78%; C/N, 2.75.

Conjugates 4d

In a series of experiments, the carrier **2d** was treated with 1.5, 1.4, 1.3, 1.2, 1.1 and 1.0 equivalents of K_2PtCl_4. the run described below, in which the Pt/NH_2 feed ratio was 1.5, is representative. A solution of **2d** (192 mg; 0.25 mmol) in water (5 ml) was saturated with nitrogen. After the addition of K_2PtCl_4 (156 mg; 0.375 mmol), the resulting solution was stirred in the dark for 22 h at ambient temperature and for 7 h at 60 °C, the pH being maintained in the conventional range of 5–6. Dialysis and freeze-drying as before afforded brownish conjugate (176 mg; 66.9%) as a water-soluble solid; η_{inh} (H_2O), 18 ml g^{-1}. Analysis: found (Cl, Pt data of two repeat runs in parentheses): C, 34.77; H, 6.71; Cl, 10.97 (12.85, 12.29); N, 13.73; Pt, 18.65% (20.04%, 18.01%); C/N, 2.95. Calcd for $(C_{34}H_{66}Cl_2N_{12}O_9Pt)_n$ [**4d** ($y=x$)]: C, 38.78; H, 6.32; Cl, 6.73; N, 15.96; Pt, 18.53%; C/N, 2.83.

Treatment of **2d** (192 mg; 0.25 mmol) in water (5 ml) with K_2PtCl_4 (145 mg; 0.35 mmol; Pt/NH_2 feed ratio 1.4) as in the preceding experiment gave 184 mg (69.9%) of light-brown water-soluble conjugate; η_{inh} (H_2O), 20 ml g^{-1}. Analysis: Found (Cl, Pt data for duplicate runs in parentheses): C, 34.78; H, 6.63; Cl, 11.23 (12.50, 11.57); N, 13.83; Pt, 20.10% (16.95%, 17.55%); C/N, 2.93. Calcd for $(C_{34}H_{66}Cl_2N_{12}O_9Pt)_n$ [**4d** ($y=x$)]: C, 38.78; H, 6.32; Cl, 6.73; Pt, 18.53%; C/N, 2.83.

The same amount of **2d**, treated with the platinum salt (135 mg; 0.325 mmol) in water (5 ml) as before ($Pt/NH_2=1.3$), was converted to water-soluble conjugate **4d** ($y=0.95x$) in a yield of 152 mg (58.5%); η_{inh} (H_2O), 18 ml g^{-1}. Analysis: Found (Cl, Pt data for duplicate run in parentheses): C, 35.54; H, 6.87; Cl, 12.82 (11.50); N, 13.99; Pt, 16.31% (18.71%); C/N, 2.96. Calcd for $C_{34}H_{65.9}Cl_{1.9}N_{12}O_{8.95}Pt_{0.95})_n$ [**4d** ($y=0.95x$)]: C, 39.31; H, 6.39; Cl, 6.48; N, 16.18; Pt, 17.84%; C/N, 2.83.

With a Pt/NH_2 feed ratio of 1.2 (0.3 mmol of K_2PtCl_4 per 0.25 mmol of **2d**) under otherwise identical conditions, a yield of 178 mg (71.5%) of light-brown water-soluble solid was obtained; η_{inh} (H_2O), 19 ml g^{-1}. Analysis: Found (Cl, Pt data for duplicate run in parentheses): C, 37.17; H, 6.10; Cl, 10.93 (12.65); N, 15.21; Pt, 15.02% (16.98%); C/N, 2.85. Calcd for $(C_{34}H_{65.6}Cl_{1.6}N_{12}O_{8.8}Pt_{0.8})_n$ [**4d** ($y=0.8x$)]; C, 40.99; H, 6.64, Cl, 5.69; N, 16.87; Pt, 15.67%; C/N, 2.83.

Analogously, a Pt/NH_2 feed ratio of 1.1 (0.275 mmol of K_2PtCl_4 per 0.25 mmol of **2d**, dissolved in 6 ml of water) gave a light-brown water-soluble conjugate in a yield of 129 mg (54.9%); η_{inh} (H_2O), 9 ml g^{-1}. Analysis: Found (Cl, Pt data for duplicate run in parentheses): Cl, 10.11 (8.56); Pt, 13.52% (11.08%). Calcd for $(C_{34}H_{65.2}Cl_{1.2}N_{12}O_{8.6}Pt_{0.6})_n$ [**4d** ($y=0.6x$)]: Cl, 4.53; Pt, 12.46%.

Finally, with 0.25 mmol each of K_2PtCl_4 and **2d** (feed ratio 1.0) dissolved in 6 ml of water, tan-brown water-soluble conjugate (135 mg; 59.3%) was obtained; η_{inh} (H_2O), 10 ml g^{-1}. Analysis: Found (Cl, Pt data for duplicate run in parentheses): Cl, 9.61 (8.81); Pt, 12.15% (9.61%). Calcd for $(C_{34}H_{65}ClN_{12}O_{8.5}Pt_{0.5})_n$ [**4d** ($y=0.5x$)]: Cl, 3.89; Pt, 10.71%.

In all experiments described in the foregoing, second fractions were collected as detailed in the preparation of **4a** ($y=x$). Yields were 20–30%, and η_{inh} ranged from 5 to 10 ml g^{-1}.

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