Selective Platination of Biologically Relevant Polyamines. Linear Coordinating Spermidine and Spermine as Amplifying Linkers in Dinuclear Platinum Complexes

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A new approach to the synthesis of novel bifunctional dinuclear platinum complexes with linear coordinating spermidine and spermine is reported. The synthetic pathway involves first the three-step selective protection of the polyamines, giving bis(trifluoroacetyl)polyamines (**1**, **4**), (*tert*-butoxycarbonyl)bis(trifluoroacetyl)polyamines (**2**, **5**), and (*tert*-butoxycarbonyl)polyamines (**3**, **6**), respectively. The platination at desired sites with activated species of *cis*- or *trans*-$[PtCl_2(NH_3)_2]$ (CDDP or TDDP, respectively) produces the BOC-protected dinuclear species $[\{cis\text{- or }trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-L})]X$ (**7**, L = BOC-spermidine, X = $(NO_3)_{0.75}Cl_{1.25}$; **9**, L = $(BOC)_2$-spermine, X = Cl_2; *cis* spermine species not isolated). Through final deprotection, three different complexes were obtained and further investigated: $[\{trans\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-spermidine-}N^1,N^8\}]Cl_3$ (**8**), $[\{trans\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-spermine-}N^1,N^{12}\}]Cl_4$ (**10**), and $[\{cis\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-spermine-}N^1,N^{12}\}]Cl_4$ (**11**). One- and two-dimensional NMR solution studies provided evidence that **11**, at physiological pH, forms an inert bis((tetraamine)platinum) species in which each Pt is chelated by a central and a terminal amino group. In contrast, complexes **8** and **10** retain their reactivity, showing only reversible formation of hydroxo bridges. The comparison of in vitro cytotoxicity data for **8**, **10**, and **11** with data for previously described bifunctional dinuclear complexes shows the enhanced activity particularly of complex **8** in the CDDP-resistant L1210 cell line. The binding of **8** and **10** to poly(dG-dC)·poly(dG-dC) is further increased and also reflected by B → Z conformational changes at lower doses.

Introduction

Dinuclear platinum complexes with bridging diamine linkers constitute a class of compounds displaying novel antitumor and DNA-binding properties.[1−3] Bifunctional "bis(platinum)" complexes of general formula $[\{cis\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-}NH_2(CH_2)_n\text{-}NH_2\}]^{2+}$ or $[\{trans\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-}NH_2(CH_2)_nNH_2\}]^{2+}$ (n = 2−6) are of particular interest because they show high activity in vitro and in vivo against tumor cell lines resistant to cisplatin (CDDP, *cis*-$[PtCl_2(NH_3)_2]$).[4−6] They have been found to be especially efficient in causing irreversible B → Z transitions in poly(dG-dC).poly(dG-dC).[7,8]

The polyamines spermidine and spermine play an essential role in normal cell growth and differentiation in eukaryotic cells.[9−11] Being protonated at physiological pH, theese polyamines have polycationic character, which provides for the capability of noncovalent interaction with negatively charged nucleic acids.[12,13] The association of polyamines and analogues with DNA can induce significant structural changes, among them being transitions of B- to Z- and A-forms of DNA.[14−16] The crystal structure of a pure-spermine Z-DNA complex has been determined, providing structural insight into the role polyamines might play as effective inducers of DNA conformational changes.[17,18] Although far from being completely understood, these structural changes are assumed to be important in the process of cell division and differentiation.[19,20]

Attention has previously been drawn to some structural similarities between dinuclear platinum complexes and the polyamines with respect to charge and separation by a lipophilic diamine backbone.[7] In optimizing the efficiency of the dinuclear platinum complexes to induce the B → Z transition, it is therefore logical to consider incorporation of ligands such as spermine and spermidine into the diamine backbone. Early

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(1) Farrell, N. *Comments Inorg. Chem.* **1995**, *16*, 373.
(2) Farrell, N. In *Adv. DNA Sequence Specific Agents* **1996**, *2*, 187.
(3) Farrell, N. In *Interaction of Anticancer Agents with Nucleic Acids*; Palumbo, M., Ed.; CRC Press: Boca Raton, FL; In Press.
(4) Farrell, N.; Qu, Y.; Hacker, M. P. *J. Med. Chem.* **1990**, *33*, 2179.
(5) Hoeschele, J. D.; Kraker, A. J.; Qu, Y.; Van Houten, B.; Farrell, N. In *Molecular Basis of Specificity in Nucleic Acid-Drug Interactions*: Pullmann, B., Jortner, J., Eds.; Kluwer Academic Press: Dordrecht, The Netherlands, 1990; pp 301−321.
(6) Farrell, N. *Cancer Invest.* **1993**, *11*, 578.
(7) (a) Johnson, A.; Qu, Y.; Van Houten, B.; Farrell, N. *Nucleic Acids Res.* **1992**, *20*, 1697. (b) Wu, P. K.; Qu, Y.; Van Houten, B.; Farrell, N *J. Inorg. Biochem.* **1994**, *54*, 207. (c) Wu, P. K.; Kharatishvili, M.; Qu, Y.; Farrell, N *J. Inorg. Biochem.* **1996**, *63*, 9.
(8) Farrell, N.; Appleton, T. G.; Qu, Y.; Roberts, J. D.; Soares-Fontes, A. P.; Skov, K. A.; Wu, P.; Zou, Y. *Biochemistry* **1995**, *34*, 15480.
(9) Pegg, A. E. *Biochem. J.* **1986**, *234*, 249.
(10) Pegg, A. E. *Cancer Res.* **1988**, *48*, 759.
(11) Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1984**, *53*, 749.
(12) Hayne, D. M.; Moreton, A. D. *J. Chem. Soc., Perkin Trans. 2* **1994**, 265.
(13) Feuerstein, B. G.; Williams, L. D.; Basu, H. S.; Marton, L. J. *J. Cell. Biochem.* **1991**, *46*, 37.
(14) Gosule, L. C.; Schellmann, J. A. *J. Mol. Biol.* **1978**, *121*, 311.
(15) Behe, M.; Felsenfeld, G. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1619.
(16) Jain, S.; Zou, G.; Sundaralingam, M. *Biochemistry* **1989**, *28*, 2360.
(17) Bancroft, D.; Williams, L. D.; Rich, A.; Egli, M. *Biochemistry* **1994**, *33*, 1073.
(18) Egli, M.; Williams, L. D.; Gao, Q.; Rich, A. *Biochemistry* **1991**, *30*, 11388.
(19) Basu, H. S.; Feuerstein, B. G.; Marton, L. J. Polyamine-DNA Interactions and Their Biological Relevance. In *Proceedings of Polyamines in the Gastrointestinal Tract*; Falk Symposium 62, Oct 6−8, 1991; Kluwer Publishing: Lancaster, England, 1992.
(20) (a) Navarro-Ranninger, C.; Amo Ochoa, P.; Perez, J. M.; Gonzalez, V. M.; Masaguer, J. R.; Alonso, C. *J. Inorg. Biochem.* **1994**, *53*, 177. (b) Navarro-Ranninger, C.; Perez, J. M.; Zamora, F.; Masaguer, J. R.; Gonzalez, V. M.; Alonso, C. *J. Inorg. Biochem.* **1993**, *52*, 37. (c) Navarro-Ranninger, C.; Zamora, F.; Pérez, J. M.; López-Solera, I.; Martínez-Carrera, S.; Masaguer, J. R.; Alonso, C. *J. Inorg. Biochem.* **1992**, *46*, 267.

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attempts to synthesize polyamine−platinum complexes gave species behaving more like a [Pt(tetraamine] complex than CDDP.[21] Well-characterized multinuclear Pt and Pd complexes of spermidine and spermine have been reported.[20] Reaction of K_2MCl_4 (M = Pd, Pt) with the free polyamines gave products distinctively different from bifunctional bis(platinum) complexes in at least two respects: (a) The central, secondary amino group(s) of the spermidine (spermine) ligand is (are) involved as well in binding to Pt or Pd, thereby forming chelates. Compared to the case of $[\{trans\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-}NH_2(CH_2)_6NH_2\}]^{2+}$, this leads to reduced flexibility of the linker and a shorter distance between the alkylating metal centers and might also have an effect on the capabilities for hydrogen-bonding with DNA. (b) The two or three polyamine-linked Pt or Pd centers themselves each have a CDDP-like structure. This implies a spectrum of DNA-binding modes different from those of bifunctional $[\{cis\text{-}$ or $trans\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-}NH_2(CH_2)_nNH_2\}]^{2+}$ complexes. With respect to platinum then, all previously reported complexes are similar to chelates such as (ethylenediamine)- or (1,2-diaminocyclohexane)platinum and chelate formation destroys the intrinsic advantages of polyamine−DNA binding such as H-bonding in defined distances along the polymer backbone. This paper reports on the incorporation of linear coordinating spermine and spermidine as amplifying linkers in dinuclear platinum complexes and the effect upon cytotoxicity and DNA-binding properties.

Experimental Section

Starting Materials and Instrumentation. Spermidine (4-azaoctane-1,8-diamine) and spermine (4,9-diazadodecane-1,12-diamine) were purchased from Fluka. *trans*-$[PtCl_2(NH_3)_2]$ (TDDP) was purchased from Johnson Matthey, and *cis*-$[PtCl_2(NH_3)_2]$ (CDDP) was prepared according to literature procedure.[22] *cis*-$[PtCl(NH_3)_2(DMF)]NO_3$ and *trans*-$[PtCl(NH_3)_2(DMF)]NO_3$ were prepared in situ by reacting CDDP and TDDP with 0.97 equiv of $AgNO_3$ in DMF solution (c = 0.05 M) at room temperature, the reaction times being 24 and 18 h, respectively. Poly(dG-dC)·poly(dG-dC) was purchased from Pharmacia, with 4.7 × 10^5 average molecular weight. Melting points were determined with a Buchi 535 instrument in open capillary tubes, and the data are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 200 MHz, a Varian Gemini 300 MHz, or a Varian Unity 500 MHz spectrometer. Chemical shifts are referenced to internal standards Me_4Si for organic solvents and TSP (2-(trimethylsilyl)propanesulfonate, sodium salt) for D_2O. ^{195}Pt NMR spectra were obtained on a Bruker 200 MHz and a Varian Gemini 300 MHz spectrometer and referenced to K_2PtCl_4 (δ = −1624 ppm) as external standard. Microanalyses were carried out by Redox snc, Cologno Monzese (MI), Italy, and Robertson Microlit Laboratories, Madison, NJ. FPLC separations were performed on a Pharmacia automated FPLC workstation. UV spectra were measured on a JASCO V-550 UV/vis spectrophotometer, and CD spectra were recorded on a JASCO J-600 spectropolarimeter using a quartz cell with a 1 cm path length. A Varian 300Z atomic absorption spectrophotometer with a GTA graphite tube atomizer was used to determine the relative binding (r_b) of platinum complex−DNA adducts. Samples were dialyzed using a 1200 MD microdialysis system from Gibco BRL Life Technologies.

2D NMR Spectroscopy. The 2D DQF-COSY and the 2D TOCSY spectra were recorded in a mixture of H_2O/D_2O (9/1) using the WET solvent suppression technique[23] at 295 K. A total of 512 t_1 increments, each with 2048 t_2 complex points, were collected with a sweep width of 7486 Hz. Each FID was the average of 32 transients. 2D ROESY spectra in D_2O were recorded at 301 K using the States/TPPI technique[24] for phase cycling. The mixing time was 500 ms. A total of 256 t_1 increments, each with 2048 t_2 complex points, were collected at a sweep width of 7994 Hz. Each FID was the average of 64 transients. The 2D data were processed using the FELIX NMR data processing package (Biosym/MSI) on a Silicon Graphics workstation.

Cytotoxicity Studies. Assays were performed as described previously.[4]

DNA Conformational Studies: Platination of DNA. A 1 OD (optical density) unit of poly(dG-dC)·poly(dG-dC) in 1xTE buffer (10 mM Tris, 1 mM EDTA, pH 7.2) was treated with various volumes of a 1 mM stock solution of the platinum complex, dissolved in 1xTE buffer immediately prior to use, to achieve a final drug concentration in a volume of 150 μL for CD measurements. Samples were incubated for 1 h at 37 °C and extensively dialyzed against 1xTE buffer at 4 °C for 2 days. DNA concentration was verified using UV spectroscopy. The quantitation of Pt−DNA binding (r_b: $[compound]_{molar}/DNA_{bp}$) was calculated on the basis of DNA concentration (molar extinction coefficient ϵ_{260} = 16 800 cm^{-1} M^{-1} per base pair), and the amount of bound Pt was measured by FAAS. Circular dichroism studies were conducted as described previously.[7]

Preparations. I. Selective Protection of Spermidine. N^1,N^8-bis(trifluoroacetyl)spermidine Trifluoroacetate (1). To a solution of spermidine (4 g, 27.5 mmol) in CH_3CN (60 mL) were added ethyl trifluoroacetate (11.4 mL, 96.2 mmol) and water (0.6 mL, 33 mmol), and the reaction mixture was heated at reflux for 3 h. The solvent was partially removed under vacuum to lower the volume of the mixture to about 20 mL. Crystallization of a white solid occurred, and after addition of CH_2Cl_2 (5 mL), the precipitate was filtered off and washed with CH_2Cl_2. The solid was dried, giving N^1,N^8-bis(trifluoroacetyl)-spermidine trifluoroacetate (**1**) (10.87 g, 87.6% yield, mp 144−146 °C), which was directly used in the next step without further purification. Anal. Calcd for $C_{13}H_{18}N_3O_4F_9$: C, 34.6; H, 4.02; N, 9.31; F, 37.89. Found: C, 34.88; H, 4.00; N, 9.45; F, 37.18.

N^4-(*tert*-Butoxycarbonyl)-N^1,N^8-bis(trifluoroacetyl)spermidine (2). Under nitrogen atmosphere, a suspension of N^1,N^8-bis(trifluoroacetyl)-spermidine trifluoroacetate (**1**, 10 g, 22.1 mmol) in TEA (67 mL) was cooled to 0 °C, and a solution of di-*tert*-butyl dicarbonate [$(BOC)_2O$, 5.3 g, 24.3 mmol] in THF (10 mL) was added. After addition, a clear yellow solution was obtained along with some gummy white solid at the bottom of the flask, and the mixture was allowed to come to room temperature. After 3 h, the yellow solution was quenched in water (200 mL) and extracted with EtOAc (3 × 100 mL). Combined organic extracts were washed with water and dried over sodium sulfate. The solvent was removed under vacuum, and the residual oil (10 g) was crystallized from 1:3 Et_2O/hexane (40 mL) to yield N^4-(*tert*-butoxycarbonyl)-N^1,N^8-bis(trifluoroacetyl)spermidine (**2**) as a white solid (9.5 g, 99% yield, mp 74 °C). Anal. Calcd for $C_{16}H_{25}N_3O_4F_6$: C, 43.94; H, 5.76; N, 9.61; F, 26.06. Found: C, 43.97; H, 5.76; N, 9.46; F, 26.21.

N^4-(*tert*-Butoxycarbonyl)spermidine−0.25-Water (3). To a solution of N^4-(*tert*-butoxycarbonyl)-N^1,N^8 -bis(trifluoroacetyl)spermidine (**2**, 2 g, 4.6 mmol) in MeOH (60 mL), cooled to 10 °C was added dropwise 0.2 N NaOH (50 mL, 10 mmol), and the mixture was allowed to come to room temperature and stirred overnight. After removal of MeOH in vacuo, the aqueous suspension was extracted with a mixture of $CHCl_3$/MeOH (9:1; 5 × 20 mL). The combined organic extracts were dried over sodium sulfate and concentrated to dryness, affording N^4-(*tert*-butoxycarbonyl)spermidine (**3**) as a yellow oil (1 g, 89%, acidimetric titration 103%). Anal. Calcd for $C_{12}H_{27}N_3O_2\cdot 0.25H_2O$: C, 57.68; H, 10.91; N, 16.82. Found: C, 60.10; H, 11.38; N, 16.62.

II. Selective Protection of Spermine. N^1,N^{12} -Bis(trifluoroacetyl)-spermine Bis(trifluoroacetate) (4). To a solution of spermine (3.2 g, 15.8 mmol) in CH_3CN (50 mL) were added ethyl trifluoroacetate (9.4 mL, 78.7 mmol) and water (0.64 mL, 35 mmol), and the reaction mixture was heated at reflux for 3 h, after which it was allowed to come to room temperature and CH_2Cl_2 (15 mL) was added. Crystallization of a white solid occurred, and the precipitate was filtered off and washed with CH_2Cl_2. The solid was dried to give N^1,N^{12} -bis-(trifluoroacetyl)spermine bis(trifluoroacetate) (**4**, 8.67 g, 88.2% yield, mp 203−206 °C), which was directly used in the next step without further purification. Anal. Calcd for $C_{18}H_{26}N_4O_6F_{12}$: C, 34.73; H, 4.21; N, 9.81; F, 36.63. Found: C, 34.79; H, 4.24; N, 9.80; F, 36.51.

(21) Tsou, K. C.; Yip, K. F.; Lo, K. W.; Ahmad, S. *J. Clin. Hematol. Oncol.* **1978**, *7*, 322.
(22) Dhara, S. C. *Indian J. Chem.* **1970**, *8*, 143.
(23) Smallcombe, S. H.; Patt, S. L.; Keifer, P. A. *J. Magn. Reson.* **1995**, *117*, 295.
(24) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286.

***N*⁴,*N*⁹-(Bis(*tert*-butoxycarbonyl)-*N*¹,*N*¹²-bis(trifluoroacetyl)spermine (5).** Under nitrogen atmosphere, a suspension of *N*¹,*N*¹² -bis-(trifluoroacetyl)spermine bis(trifluoroacetate) (**4**, 8.67 g, 13.9 mmol) in TEA (43 mL) was cooled to 0 °C, and a solution of di-*tert*-butyl dicarbonate [$(BOC)_2O$, 6.25 g, 27.8 mmol] in THF (15 mL) was added. After 3 h, the obtained yellow solution was quenched in water (150 mL) and extracted with EtOAc (3 × 50 mL). Combined organic extracts were washed with water and dried over sodium sulfate. The solvent was removed under vacuum, and the residual oil (9 g) was crystallized from Et_2O (45 mL) to yield *N*⁴,*N*⁸-bis(*tert*-butoxycarbonyl)-*N*¹,*N*¹²-bis(trifluoroacetyl)spermine (**5**) as a white solid (7.6 g, 92% yield, mp 104−106 °C). Anal. Calcd for $C_{24}H_{40}N_4O_6F_6$: C, 48.48; H, 6.78; N, 9.42; F, 19.17. Found: C, 48.52; H, 6.80; N, 9.48; F, 19.40.

***N*⁴,*N*⁹-Bis(*tert*-butoxycarbonyl)spermine−1.25-Water (6).** To a solution of *N*⁴,*N*⁹-bis(*tert*-butoxycarbonyl)-*N*¹,*N*¹²-bis(trifluoroacetyl)-spermine (**5**, 3.3 g, 5.5 mmol) in MeOH (105 mL) cooled at 10 °C was added dropwise 0.2 N NaOH (60 mL, 12 mmol), and the mixture was allowed to come to room temperature and stirred overnight. After the removal of MeOH in vacuo, the aqueous suspension was extracted with a $CHCl_3$/MeOH mixture (9:1; 6 × 50 mL). The combined organic extracts were dried over sodium sulfate and concentrated to dryness, affording *N*⁴,*N*⁹-bis(*tert*-butoxycarbonyl)spermine (**6**) as a pale yellow oil (1.33 g, 60% yield, and acidimetric titration 98%). Anal. Calcd for $C_{20}H_{42}N_4O_4 \cdot 1.25H_2O$: C, 56.73; H, 10.62; N, 13.05. Found: C, 56.49; H, 10.57; N, 13.18.

III. Platinum Complexes of Spermidine. [{*trans*-PtCl(NH₃)₂}₂-{*μ*-*N*⁴-(*tert*-butoxycarbonyl)spermidine-*N*¹,*N*⁸}](NO₃)₀.₇₅Cl₁.₂₅ (7). To a solution of *trans*-$[PtCl(NH_3)_2(DMF)]NO_3$ (prepared in situ from 18.34 mmol of TDDP and $AgNO_3$) in 370 mL of DMF cooled to −20 °C was added 2.14 g (8.37 mmol) of *N*⁴-(*tert*-butoxycarbonyl)spermidine−0.25-water (**3**) in 50 mL of DMF over 10 min. Stirring was continued for 3 h at −20 °C and then for 1 h at room temperature. The solution was then evaporated to 100 mL volume under high vacuum (T < 30 °C). Charcoal (1 g) was added and the suspension was stirred for 10 min at room temperature; the solid filtered off, and the filtrate was evaporated to dryness. The residue was stirred in 100 mL of ether/acetone (1:1 v/v) overnight. After filtration, the solid was stirred in 400 mL of MeOH for 2 h. Undissolved yellow solid was filtered off. The filtrate was evaporated to a volume of 70 mL, and 5 mL of a 5 M LiCl solution in MeOH was added. Immediately, a white solid precipitated. The mixture was kept overnight at −18 °C, and the solid was filtered off, washed twice with 10 mL of MeOH and twice with 20 mL of acetone and ether, and dried in vacuo to afford **7** (5.34 g, 74% yield). Anal. Calcd for $C_{12}H_{39}N_{7.75}O_{4.25}Cl_{3.25}Pt_2$: C, 16.65; H, 4.55; N, 12.54; Cl, 13.31. Found: C, 16.47; H, 4.57; N, 12.36; Cl, 13.19.

[{*trans*-PtCl(NH₃)₂}₂{*μ*-spermidine-*N*¹,*N*⁸}]Cl₃ (8). To a suspension of 0.580 g (0.665 mmol) of **7** in 25 mL of MeOH was added 6 mL of concentrated HCl, and the mixture was stirred for 42 h at room temperature. The supernate was pipetted off, and the solid was stirred in 100 mL of acetone overnight, after which it was filtered off, washed four times with 15 mL of acetone, and pumped dry to give **8** (484 mg, 93% yield). Anal. Calcd for $C_7H_{32}N_7Cl_5Pt_2$: C, 10.75; H, 4.13; N, 12.54; Cl, 22.70. Found: C, 10.95; H, 4.00; N, 12.32; Cl, 22.48.

IV. Platinum Complexes of Spermine. [{*trans*-PtCl(NH₃)₂}₂{*μ*-*N*⁴,*N*⁹-bis(*tert*-butoxycarbonyl)spermine-*N*¹,*N*¹²}]Cl₂ (9). To a solution of 6.00 mmol of *trans*-$[PtCl(NH_3)_2(DMF)]NO_3$ (prepared in situ from TDDP and $AgNO_3$) in 120 mL of DMF cooled to −20 °C was added 1.00 g (2.50 mmol) of **6** in 20 mL of DMF over 10 min. The solution was stirred for 3 h at −20 °C and then for 1 h at room temperature. One gram of activated carbon was added to the solution, and stirring was continued for 30 min. After filtration (sintered-glass filter with Celite pad), the clear, slightly yellowish solution was concentrated under high vacuum to 30 mL. Addition of 100 mL of acetone followed by 100 mL of ether caused precipitation of a light yellowish solid, which was filtered off, dried, and redissolved in 100 mL of MeOH containing 220 mg of NaCl (stirring at room temperature for 4 h). The mixture was kept at −18 °C for 2 h, after which the precipitate was filtered off and dried. The solid was dissolved in 100 mL of MeOH, 0.5 g of activated carbon was added, and the mixture was stirred for 30 min, followed by filtration. The filtrate was concentrated to 10 mL by rotary evaporation and kept overnight at −18 °C. The white precipitate was filtered off, washed with acetone and ether, and dried under vacuum. Addition of 50 mL of ether to the filtrate gave a second crop of the product **9** (590 mg, 24% yield). The compound was characterized by ^{1}H NMR and then directly used in the next step without further purification.

[{*trans*-PtCl(NH₃)₂}₂{*μ*-spermine-*N*¹,*N*¹²}]Cl₄ (10). A solution of 390 mg (0.389 mmol) of **9** in a mixture of 25 mL of MeOH, 25 mL of H_2O, and 10 mL of concentrated HCl was stirred at room temperature for 4 h. The white precipitate was filtered off, washed with acetone (4 × 15 mL) and ether (15 mL), and dried in vacuo to give compound **10** (275 mg, 81% yield). Anal. Calcd for $C_{10}H_{40}N^8Cl_6Pt_2$: C, 13.72; H, 4.61; N, 12.80; Cl, 24.30. Found: C, 13.11; H, 4.46; N, 12.34; Cl, 24.22.

[{*cis*-PtCl(NH₃)₂}₂{*μ*-spermine-*N*¹,*N*¹²}]Cl₄ (11). To a solution of 4.8 mmol of *cis*-$[PtCl(NH_3)_2(DMF)]BF_4$ (prepared in situ from CDDP and $AgBF_4$) in 100 mL of DMF cooled to 0 °C was added 897.8 mg (2.23 mmol) of **6** in 10 mL of DMF over 10 min. The mixture was stirred for 20 h at room temperature. The solution was then evaporated to a 10 mL volume and 100 mL of ether was added, resulting in precipitation of an oily material. The solvent was decanted, and the oily residue was dissolved in 150 mL of MeOH; 25 mL of concentrated. HCl was then added to achieve the deprotection of the spermine linker. The solution was stirred for 42 h at room temperature. During this time, a solid started to precipitate. The solid was filtered off, dried, and redissolved in 2 mL of H_2O, and the solution was loaded onto a Sephadex G10 column (Pharmacia XK 16/70) and eluted with H_2O. The separation was monitored by UV absorption and conductivity traces; suitable fractions were pooled and evaporated to dryness. The solid was then stirred for 24 h in 100 mL of acetone/ether (1:1 v/v), filtered off, and dried in vacuo to give **11** (654 mg, 16% yield). Anal. Calcd for $C_{10}H_{40}N_8Cl_6Pt_2$: C, 13.72; H, 4.61; N, 12.80; Cl, 24.30. Found: C, 13.86; H, 4.25; N, 12.58; Cl, 24.54.

Solution Studies. I. Complexes 8 and 10 in Aqueous Solution at pH 7.3. A 7.81 mg (0.01 mmol) sample of **8** or 8.75 mg (0.01 mmol) of **10** was dissolved in 1 mL of phosphate buffer[21] [KH_2PO_4/Na_2HPO_4 , 0.15 M in D_2O (Cambridge Scientific)] at pH 7.3, and the solution was kept at 37 °C for 2 days.

II. Complex 11 in Aqueous Solution at pH 7.3. For ^{1}H NMR spectroscopic measurements, 17 mg (0.02 mmol) of **11** was dissolved in 1 mL of phosphate buffer[25] (KH_2PO_4/Na_2HPO_4, 0.067 M in D_2O or H_2O/D_2O, 9/1) at pH 7.3, and the solution was kept at 37 °C for 2 days. For a second set of samples, 87.5 mg (0.1 mmol) of **11** was dissolved in 25 mL of phosphate-buffered saline in H_2O (Sigma; 0.01 M phosphate; 0.12 M NaCl; 0.0027 M KCl; $pH_{measured}$ 7.3), and the solution was kept at 37 °C for 2 days. The samples were then evaporated to dryness, redissolved in 1 mL of D_2O, and used for ^{1}H and ^{195}Pt NMR spectroscopic measurements. The obtained ^{1}H NMR spectra for both sets of conditions were identical.

Results and Discussion

Selective Protection of Spermidine and Spermine Linkers and Syntheses of Spermidine and Spermine-Bridged Bis-(platinum) Complexes. The polyamines spermidine and spermine are ligands with multiple potential binding sites for coordinatively unsaturated platinum complexes.[20,21] In order to prevent the metalation of both primary and secondary amino groups of spermidine and spermine, a selective protection of the secondary groups was necessary. The general synthetic pathway for the polyamine protection and the preparation of the Pt complexes applied in the present work is summarized (for spermidine as an example) in Figure 1.

For the first step, the selective protection of the primary amines in the presence of secondary ones, a procedure published in two very recent papers using ethyl trifluoroacetate was followed.[26] The subsequent BOC protection of the secondary amino groups in both diacylated spermidine and spermine and the hydrolysis of trifluoroacetamides were achieved with

(25) Walbum, L. E. *Biochem. Z.* **1920**, *107*, 219.

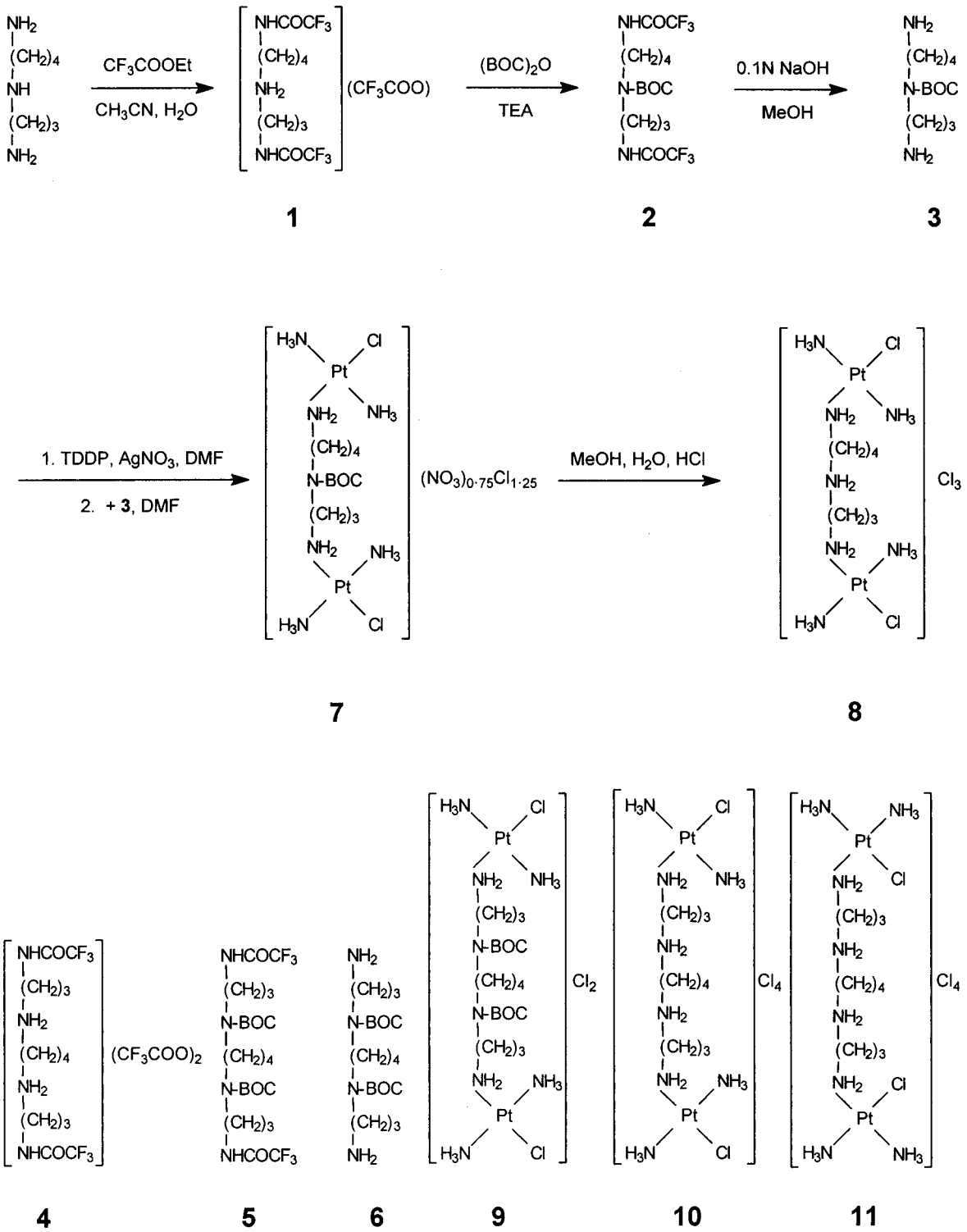


Figure 1. Selective protection of spermidine and preparation of the bridged bis(platinum) complexes **7** and **8**. The corresponding scheme using spermine gives the analogous protected spermine linkers **4**−**6** and Pt complexes **9**−**11**.

standard methods.[27] The syntheses of the platinum complexes were carried out by reacting the monoactivated solvent species *trans*-[PtCl(NH$_3$)$_2$(DMF)]NO$_3$ or its *cis* isomer with the BOC-protected polyamines. The deprotection in acidic media afforded the final [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-spermidine-N^1,N^8}]Cl$_3$ (**8**), [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-spermine-N^1,N^{12}]Cl$_4$ (**10**), and [{*cis*-PtCl(NH$_3$)$_2$}$_2${μ-spermine-N^1,N^{12}}]Cl$_4$ (**11**). Table 1 lists ^{1}H NMR chemical shifts for spermidine and spermine linkers **1**−**6** and ^{1}H and ^{195}Pt NMR chemical shifts for Pt complexes **7**−**11**. The numbering scheme used for subsequent assignment of signals is shown in Chart 1.

The spermidine and spermine complexes all show four major sets of ^{1}H NMR signals for the methylene protons of the alkyl chains. An upfield shift for the H$_{b,b'}$ signals of **7**, **9**, and **11** compared to the uncomplexed linkers is consistent with binding of Pt only to the primary, terminal amino groups while the

(26) (a) O'Sullivan, M.; Dalrymple, D. M. *Tetrahedron Lett.* **1995**, *36*, 3451 and references cited therein. (b) Xu, D.; Repic, O.; Blacklock, T. J. *Tetrahedron Lett.* **1995**, *36*, 7357 and references cited therein.

(27) (a) Saari, W. S.; Schwering, J. E.; Lyle, P. A.; Smith, S. J.; Engelhardt, E. L. *J. Med. Chem.* **1990**, *33*, 97 and references therein. (b) Krapcho, A. P.; Kuell, C. S. *Synth Commun.* **1990**, *20*, 2559 and references therein. (c) Acton, E. M.; Tong, G. L. *J. Med. Chem.* **1981**, *24*, 669.

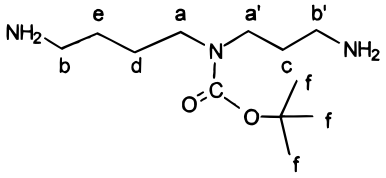
(28) No spectral evidence for the presence of different diastereoisomers was found.

Table 1. ^{1}H NMR Chemical Shifts of Nonexchangeable Protons in Spermidine (**1**−**3**) and Spermine (**4**−**6**) Ligands and ^{1}H NMR and ^{195}Pt NMR Chemical Shifts of Pt Complexes **7**−**11**[a]

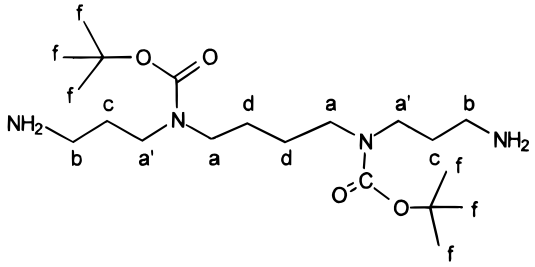
compd	δ(^{1}H), ppm	δ(^{195}Pt), ppm
1[b]	3.3 (m, $H_{b,b'}$); 2.9 (m, $H_{a,a'}$); 1.8 (m, H_c); 1.5 (m, $H_{d,e}$)	
2[b]	3.1 (m, $H_{a,a'}$, $H_{b,b'}$); 1.7 (m, H_c); 1.4 (m, $H_{d,e}$, H_f)	
3[c]	3.3 (2m, $H_{a,a'}$); 3.01 ($H_{b,b'}$); 1.92 (q, H_c); 1.64 (m, $H_{d,e}$); 1.46 (s, H_f)	
4[b]	3.3 (m, $H_{b,b'}$); 2.9 (m, $H_{a,a'}$); 1.85 (m, H_c); 1.6 (m, $H_{d,e}$)	
5[b]	3.1 (m, $H_{a,a'}$, $H_{b,b'}$); 1.7 (m, H_c); 1.4 (m, $H_{d,e}$, H_f)	
6[c]	3.3 (m, $H_{a,a'}$); 3.0 (t, $H_{b,b'}$); 1.9 (q, H_c); 1.55 (br s, $H_{d,e}$); 1.45 (s, H_f)	
7[c]	3.30 (m, $H_{a,a'}$); 2.71 (t, $H_{b,b'}$); 1.91 (m, H_c); 1.67 (m, $H_{d,e}$); 1.50 (s, H_f)	−2424
8[c]	3.12 (2t, $H_{a,a'}$); 2.77 (2t, $H_{b,b'}$); 2.11 (q, H_c); 1.77 (m, $H_{d,e}$)	−2434
9[c]	3.29 (m, $H_{a,a'}$); 2.69 (t, $H_{b,b'}$); 1.89 (q, H_c); 1.53 (m, $H_{d,e}$); 1.46 (s, H_f)	−2424
10[c]	3.13 (2t, $H_{a,a'}$); 2.88 (t, $H_{b,b'}$); 2.11(q, H_c); 1.78 (m, $H_{d,e}$)	−2426
11[c]	3.16 (2t, $H_{a,a'}$); 2.78 (t, $H_{b,b'}$); 2.14 (q, H_c); 1.79 (m, $H_{d,e}$)	−2423

[a] Proton assignment according to Chart 1. [b] DMSO-d_6. [c] D_2O; pD ≤7. All ^{195}Pt in D_2O.

Chart 1



N4-BOC-spermidine



N4,N9-BOC-spermine

signals for the secondary amino functions (particularly in **7** and **9**) remain essentially unchanged (most downfield signal).

Solution Behavior of Complexes 8, 10, and 11. In aqueous solution (pD 4.7−5.1), the ^{1}H NMR spectra of complexes **8**, **10**, and **11** do not change over a period of up to 2 weeks at 37 °C. When these complexes are dissolved in a phosphate buffer (pD 7.3−7.4) however, changes can be observed within 2 days. In this case, the complex having a *cis* configuration of the Cl^- leaving group relative to the polyamine linker (**11**) behaves differently from the *trans* complexes **8** and **10**.

I. Complex 11. After 2 days at 37 °C and pD 7.3, the ^{195}Pt NMR resonance of **11** (D_2O; δ = −2423 ppm), typical for a $PtClN_3$ environment, disappears and is replaced by a new signal (D_2O; δ = −2708 ppm), indicating that a complex with a PtN_4 environment is formed. This new species **12** displays an ^{1}H NMR spectrum with a complicated coupling pattern for the CH_2 protons (Figure 2). The increased complexity is evident, and six multiplets are observed for the methylene protons (D_2O; δ (ppm) = 1.80, 1.96, 2.15, 2.79, 2.98, 3.14), as well as five sets for the NH protons (H_2O/D_2O; δ (ppm) = 6.11, 5.29, 4.58, 4.15, 4.11; not shown). Chart 2 schematically presents the proposed structure of **12**.

In order to obtain further structural information on **12** and resolve the complex coupling, several two-dimensional NMR

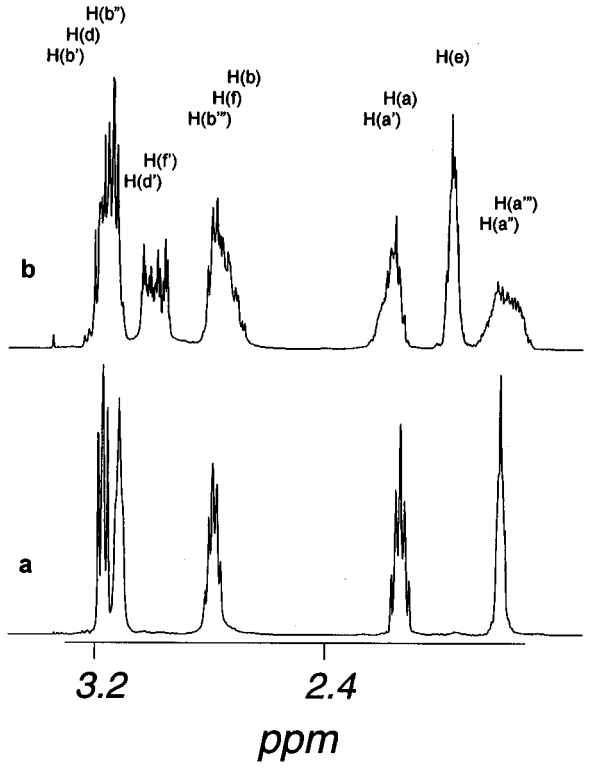
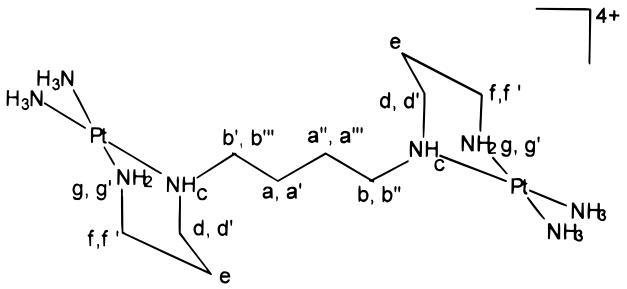


Figure 2. Sections of one-dimensional 500 MHz ^{1}H NMR spectra of **11** (a) and **12** (b) in D_2O. Note: The high magnetic field strength separates the two triplets of **11** for H_a and $H_{a'}$ (Chart 1).

Chart 2



experiments were performed. Sections of the 2D DQF-COSY, 2D TOCSY (both in H_2O/D_2O; 9/1), and 2D ROESY (in D_2O) spectra of **12** are shown in Figure 3. The assignment of the protons of the spermine linker follows Chart 2. It is evident that, upon coordination of the two N_c nitrogens to Pt, the former become chiral centers. It can be expected that both enantiomers and the meso form are present in equal amounts since there is no obvious driving force for chiral discrimination.[29]

From the 2D spectra a total of 16 inequivalent protons can be distinguished within the framework of the total 26 spermine protons, using NH resonances as starting points. The amino and ammine signals were assigned by connectivity patterns found in the COSY and TOCSY spectra. The downfield signal at 6.11 ppm of the secondary amino proton H_c (Chart 2) shows coupling to H_b, $H_{b''}$ and $H_{d'}$, Figure 3a. Likewise, the strong cross-peaks between the two amino resonances at 5.29 and 4.58 ppm confirm the assignment to the two protons $H_{g/g'}$ of the primary amino groups. The inequivalent protons of the Pt−NH_3 groups (4.15 and 4.11 ppm) exchange fast even in H_2O/D_2O and are not detected in the COSY spectrum.

(29) Lim, M. C.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1976**, *38*, 1911. Martin, R. B. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209, American Chemical Society: Washington, DC, 1983; p 231.

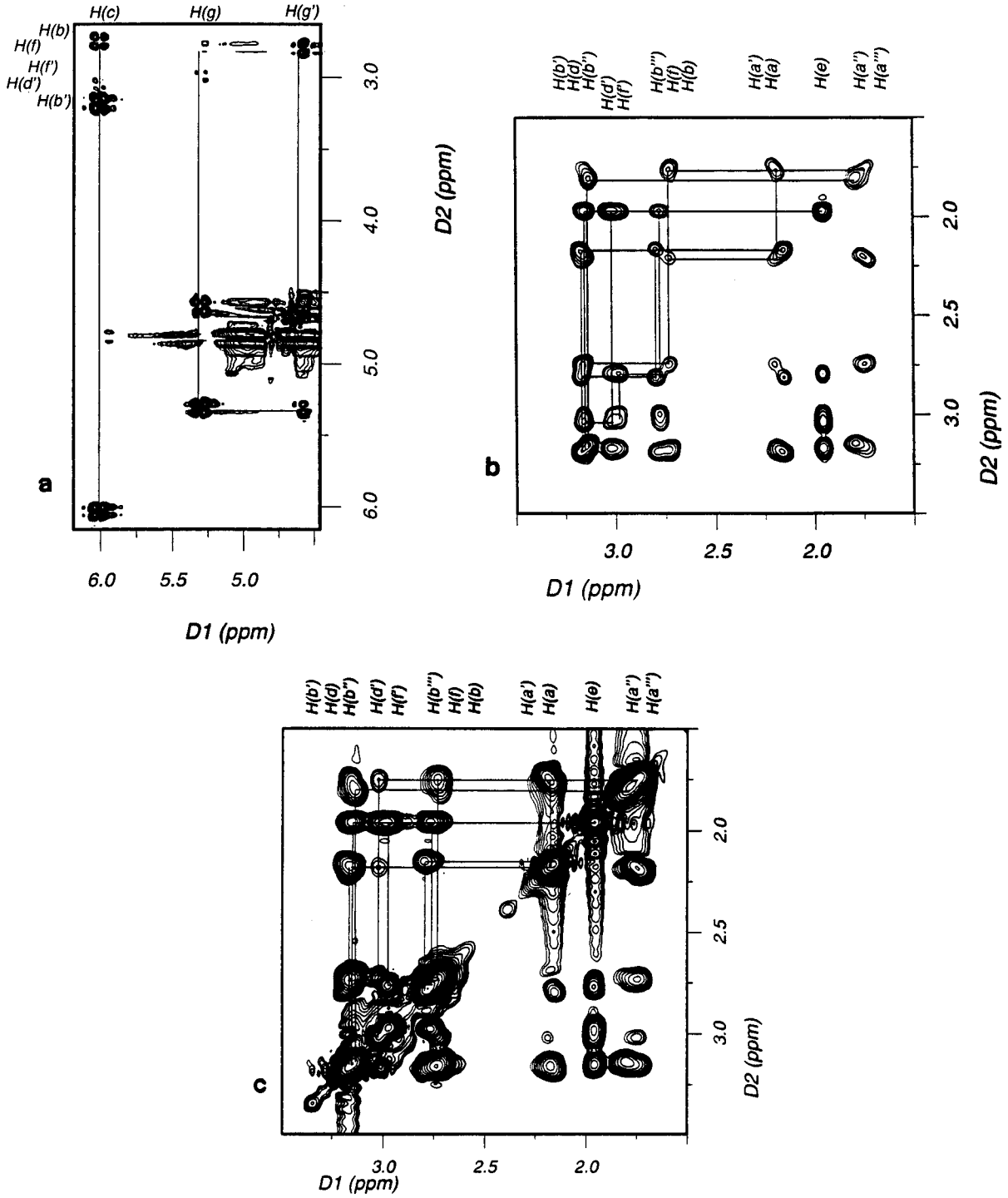


Figure 3. Sections of 2D DQF-COSY (a), TOCSY (b), and ROESY (c) spectra of species **12**. The TOCSY spectrum separates eight protons of the spermine butylene chain that are interconnected through bonds.

The methylene protons of the central butylene and the 1,3-propanediyl chains appear at chemical shifts between 3.2 and 1.7 ppm. The 2D TOCSY and ROESY spectra (Figure 3b,c) allow us to allocate connectivities and through-space coupling. The propanediamine protons in **12** are part of two six-membered rings. These two rings appear to be magnetically equivalent. $H_{d/d'}$, $H_{f/f'}$, and $H_{g/g'}$ are diastereotopic protons and therefore give separated signals, while the central $-CH_2-$ units of the propanediamine rings (H_e; with connectivity to $H_{d,d'}$ and $H_{f,f'}$; Figure 3b) give only one sharp peak in the 1D NMR spectrum (Figure 2) presumably due to conformational flexibility of the ring. Combination of TOCSY and ROESY experiments (Figure 3a,b) gave us the proton assignments of the individual $-CH_2-$ units in the butanediamine part of the linker in **12**. The four methylene groups of the central butylene chain are all prochiral. Therefore one would expect at least four proton signals assuming the presence of a plane of symmetry (*R,S* isomer) or C_2 symmetry axis (*R,R* and/or *S,S*). We actually do observe eight different proton resonances that are interconnected through bonds. The inequivalence of the pairs of $H_{a/a'}/H_{a''/a''}$ and $H_{b/b''}/H_{b'/b'''}$ protons could indicate an overall conformation of the molecule that decreases its symmetry.[28] Some chemical shift differences (e.g., $\Delta\delta = 0.7$ ppm for $H_{g/g'}$ and $\Delta\delta = 0.4$ ppm for $H_{b/b'}$) are remarkably large and reflect the unique stereochemical arrangement found in **12**.

In summary, the NMR results show that **11** undergoes an elimination reaction at neutral pH with Pt coordinating to the secondary amino nitrogens:

Scheme 1

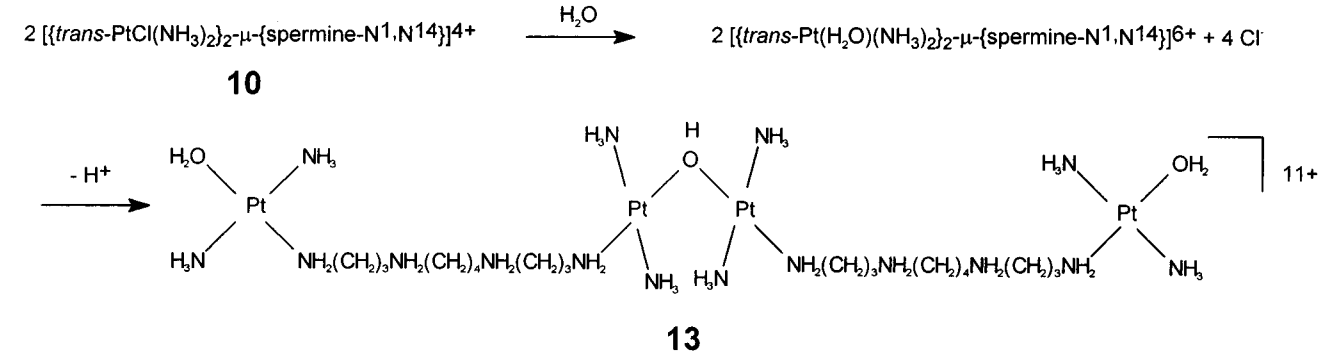


Table 2. In Vitro Cytotoxicity Data (72 h Drug Exposure) for Bis(platinum) Complexes and CDDP in Murine L1210 Leukemia Cell Lines Sensitive (L1210/0) and Resistant (L1210/DDP) to Cisplatin

complex	ID_{50}, μM		
	L1210/0	L1210/DDP	RF[a]
CDDP	0.31	8.9	29
[{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-NH$_2$(CH$_2$)$_4$-NH$_2$}]-(NO$_3$)$_2$	3.5	0.87	0.25
[{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-NH$_2$(CH$_2$)$_6$-NH$_2$}]-(NO$_3$)$_2$	0.43	0.10	0.23
[{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-spermidine-N^1,N^8}]-Cl$_3$ (**8**)	0.41	0.02	0.05
[{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-spermine-N^1,N^{12}}]-Cl$_4$ (**10**)	0.60	0.14	0.23
[{*cis*-PtCl(NH$_3$)$_2$}$_2${μ-spermine-N^1,N^{12}}]Cl$_4$ (**11**)	50	51	1

[a] Resistance factor (RF) defined as ID_{50}(resistant)/ID_{50}(sensitive).

$$[\{cis\text{-PtCl(NH}_3)_2\}_2\{\mu\text{-spermine-}N^1,N^{12}\}]^{4+} \rightarrow$$
11
$$[\{cis\text{-Pt(NH}_3)_2\}_2\{\mu\text{-spermine-}N^1,N^4,N^9,N^{12}\}]^{4+} + 2\text{HCl}$$
12

In this pH region, several units lower than the expected pK_a of a secondary amine, a fraction of the free amino groups of the spermine linker must be present in their deprotonated form (R_2NH; possibly concerted deprotonation by Pt−OH species). The nucleophilic attack of the amine on Pt is followed by release of Cl^- (or H_2O). The driving force for this process is the formation of two chelate rings in the final product. The reaction product **12** is a bis((tetraamine)platinum) cation that is no longer able to covalently bind to DNA.

II. Complexes 8 and 10. When **8** and **10** are dissolved in a phosphate buffer (pD 7.3) and the solutions are kept at 37 °C for 2 days, the ^{1}H NMR spectra show the appearance of one new multiplet. The ^{1}H NMR spectrum of the spermine complex **10** dissolved in D_2O (pD ∼5) and the spectrum of a new species **13** in buffered solution (pD 7.3) are presented in Figure 4. The latter spectrum contains an additional triplet at 2.70 ppm ($H_{b'}$), slightly upfield of the original signal for H_b (see Chart 1 and Table 1). All other resonances (H_a, H_c, H_d) remain unchanged. The ratio of the two triplets ($H_b/H_{b'}$ ∼2/3) does not change further over time. After adjusting the pD from neutral to pD 1.9 by addition of DCl and keeping the sample at 37 °C for 1 day, we observe that the ^{1}H NMR spectrum is identical with that of **10**. Only one triplet at 2.80 ppm is present by then. These results, which are observed for both complex **8** and **10**, are interpreted in terms of hydrolysis followed by formation of hydroxo-bridged oligomers of various sizes; e.g., see Scheme 1. This process, well-known for CDDP[30] and dinuclear platinum complexes,[30] takes place at neutral pH, close to the expected pK_a(s) of the aqua ligand(s). It can be reversed by lowering the pH or through attack by and substitution with other (e.g.,

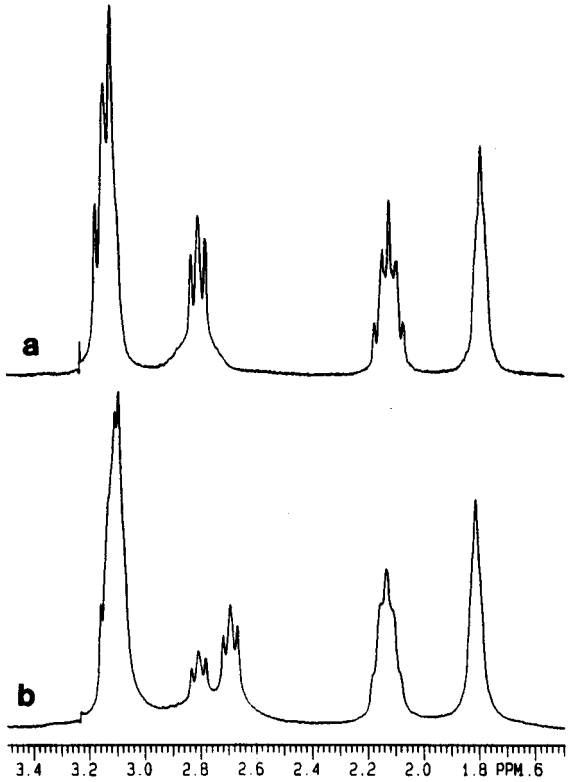


Figure 4. (a) ^{1}H NMR spectrum of **10** dissolved in D_2O (pD 5). (b) ^{1}H NMR spectrum of species **13** resulting from complex **10**, dissolved at pD 7.3, after 2 d heating at 37 °C.

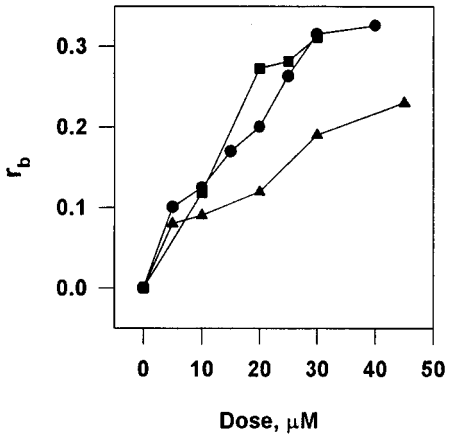
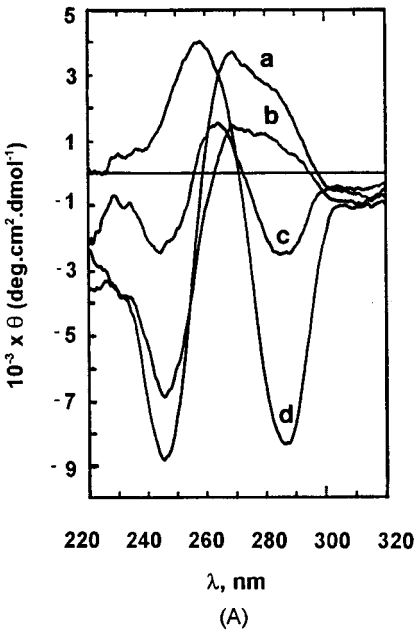


Figure 5. Quantitation of Pt−DNA binding (r_b) versus dose (μM) of the bis(platinum) complex: ●, compound **10**; ■, compound **8**; ▲, [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-NH$_2$(CH$_2$)$_4$NH$_2$}](NO$_3$)$_2$.

(30) Qu, Y.; Farrell, N. *J. Inorg. Biochem.* **1990**, *40*, 255.



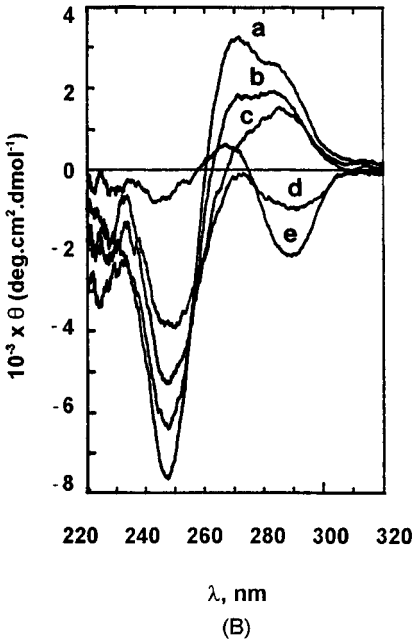


Figure 6. Circular dichroism spectra of poly(dG-dC)·poly(dG-dC) modified by complex **10** (A) and complex **8** (B). A: (a) no drug, (b) 10 μM, (c) 15 μM, (d) 20 μM, corresponding to $r_b = 0$, 0.12, 0.17, 0.20. B: (a) no drug, (b) 10 μM, (c) 20 μM, (d) 25 μM, (e) 30 μM, corresponding to $r_b = 0$, 0.12, 0.27, 0.28, 0.31.

N-donor) nucleophiles. Therefore covalent binding of **8** and **10** to DNA should not significantly be affected.

Cytotoxicity Studies. In order to assess the antitumor activity of the newly prepared polyamine complexes, they were tested in murine L1210 leukemia cells both sensitive and resistant to CDDP. Results for complexes **8**, **10**, and **11** are summarized in Table 2 and compared to data found for CDDP and the diamine-linked complexes [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-NH$_2$(CH$_2$)$_n$NH$_2$}](NO$_3$)$_2$ (n = 4, 6).[5]

The data show that all complexes except **11** have high cytotoxic activity in cisplatin-sensitive cells (L1210/0), comparable to CDDP. More striking, however, is the remarkable activity of **10** and particularly **8** in the resistant cell line (L1210/DDP). Few, if any, preclinical Pt drugs show such consistently low resistance factors.

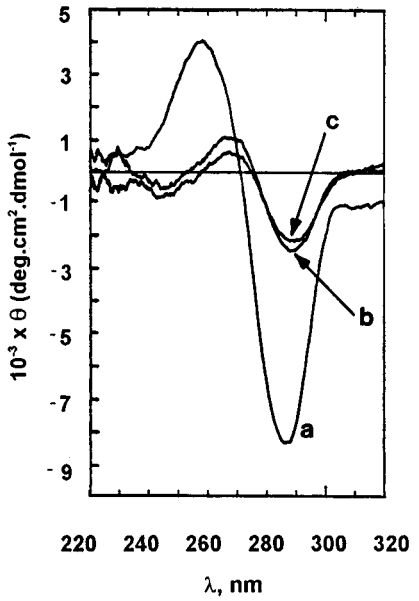


Figure 7. Circular dichroism spectra of poly(dG-dC)·poly(dG-dC) treated with (a) 20 μM ($r_b = 0.20$) complex **10**, (b) 30 μM ($r_b = 0.31$) complex **8**, and (c) 45 μM (r_b = 0.23) [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-NH$_2$(CH$_2$)$_4$NH$_2$}](NO$_3$)$_2$.

The B → Z DNA Transition of Poly(dG-dC)·Poly(dG-dC) upon Treatment with 8 and 10. The quantitation of Pt−DNA binding (r_b) as shown in Figure 5 exhibits some interesting differences between the polyamine and one of our previously described alkanediamine-linked complexes. Upon replacement of butanediamine by spermine and spermidine, increased binding of the compound to DNA at identical doses occurs. This demonstrates that DNA binding affinity is enhanced through hydrogen-bonding interactions and charge effects between the linker backbone and DNA.

Figure 6 shows the changes in the CD spectra of poly(dG-dC)·poly(dG-dC) when treated with increasing amounts of the Pt complexes. Both bis(platinum) complexes **8** and **10** are remarkably efficient at inducing the B → Z conformational changes in the polynucleotide in a dose-dependent manner. The spectra are characterized by a negative band at 290 nm and a positive band at 255−260 nm at doses of 30 μM for **8** (r_b = 0.311) and 20 μM for **10** ($r_b = 0.20$). As evident from these data, the spermine complex **10** is more efficient in converting B-DNA to Z-DNA than the spermidine analog **8**. It was found previously that spermine induces B → Z transitions in poly(dG-me^{5}dC)·poly(dG-me^{5}dC) with heating to 60 °C.[31] Combination of the linker effects (charge, hydrogen-bonding, and hydrophobic backbone effects) with covalent binding abilities of the Pt complex results in greater efficiency at inducing this type of conformational change. The B → Z transition of poly(dG-dC)·poly(dG-dC) is not complete at 30 μM concentration of compound **8**. However, at higher doses of **8**, precipitation of DNA occurs. In order to compare the linker effects, the maximum "Z-character" DNA CD spectra induced by bis(platinum) complexes are plotted in Figure 7 for the three different linkers (butanediamine, spermine, and spermidine). The CD spectra of the platinated poly(dG-dC)·poly(dG-dC) are not identical for all complexes, implying that the Pt−DNA adducts are structurally somewhat distinct. While the butanediamine- and the spermidine-linked complexes produce adducts giving very similar CD spectra, the spermine complex **10** causes changes that are reflected in CD patterns more similar to that

(31) Basu, H. S.; Schwietert, H. C. A.; Feuerstein, B. G.; Marton, L. J. *Biochem. J.* **1990**, *269*, 329.

of "classical Z-form DNA".[7] Furthermore, the irreversible nature of the B → Z transition noted previously appears to be a property shared also by the spermine and spermidine complexes.

Summary and Conclusions. Site-specific protected polyamine linkers were used in a new synthetic approach for dinuclear platinum complexes with linear coordinated spermidine and spermine. NMR solution studies have provided evidence that complex **11**, because of its geometry, forms an inert bis((tetraamine)platinum) species at physiological pH, while complexes **8** and **10** retain their reactivity under these conditions.

The introduction of biologically relevant polyamines into the structure of first-generation bifunctional bis(platinum) complexes has significant impact on their DNA-binding properties (increased r_b) and especially on their cytotoxic activity. The DNA conformational studies confirm that complexes **8** and **10** induce B to Z conformational changes at lower doses because of increased binding and higher charges as compared to diamine-bridged bis(platinum) complexes. The obtained CD spectroscopic information raises the question as to how dinuclear (polyamine) Pt complexes bind to DNA and induce Z-forms. Recently obtained data from 2D NMR and molecular modeling studies showed that, upon binding of a dinuclear Pt complex to GpG[32] and TGGT,[33] the 3′-guanine assumes a *syn* conformation of the base relative to the sugar, which is not observed in intrastrand adducts of CDDP. This conformation is a prerequisite for induction of left-handed forms in alternating cytosine−guanine sequences. The structural differences in the platinum complex−DNA adducts formed by CDDP and bis(platinum) complexes may help to explain the differences in their antitumor activity.

The crystal structure of the pure-spermine form of Z-DNA provided valuable insight into the polymorphism of modes of interactions of the cation with DNA.[17,18] Similarly, more detailed structural information on cross-links of dinuclear platinum complexes (especially with the new polyamine linkers) leading to left-handed DNA forms is highly desirable. Efforts in this direction are underway.

Acknowledgment. This work was supported by a grant from the American Cancer Society (ACS-DHP2E) and by Boehringer Mannheim Italia. We thank Dr. M. Kharatishvili for obtaining some of the CD spectra and J. Peroutka for technical assistance in obtaining cytotoxicity data.

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(32) Qu, Y.; Bloemink, M. J.; Reedijk, J.; Hambley, T. W.; Farrell, N. *J. Am. Chem. Soc.* **1996**, *118*, 9307.
(33) Kasparková, J.; Mellish, K. J.; Qu, Y.; Brabec, V.; Farrell, N. *Biochemistry*, **1996**, *35*, 16705.