11 (0.0015% wet weight) crystallized from hexane–ethyl acetate (2:1) and was recrystallized from methylene chloride to yield colorless needles, mp 204–206 °C ($C_{15}H_{12}BrN_3$, HREIMS Δ 2.0 mmu). The mother liquid on C_{18} reversed-phase MPLC (MeOH:H_2O, 9:1) gave **12** (0.0011%, yellow powder, mp 140–142 °C, $C_{15}H_{12}BrN_3$, HREIMS Δ 0.7 mmu) and **13** (0.0010%, colorless powder, mp 153–155 °C, $C_{15}H_{13}N_3$, HREIMS Δ 1.5 mmu). The UV spectra of **11–13**[4] argue the presence of a β-carboline chromophore.[5] Signals at 176.3–176.8 ppm in the ^{13}C spectra of **11–13**[4] are assignable to an imino carbon (C=N)[13] and deuterium-exchangeable signals at 10.9–11.0 ppm to an NH proton (Table II). Reduction of **11** (FABMS, M + H, *m/z* 314, Br) with sodium borohydride in methanol gave amine **14** (FABMS, M + H, *m/z* 316, Br), which was acetylated to **15** (FABMS, M + H, *m/z* 358, Br; NCO, 1650 cm^{-1}). The UV spectrum of **15**[4] is nearly identical with that of the β-carboline harman.[5,14] The ^1H NMR spectra of **11–13** (Table III) establish the substitution pattern as a β-carboline skeleton,[7,10,14,15] in which the benzenoid ring is unsubstituted in **13** but substituted in **11** and **12** by bromine at C-7 and C-6, respectively.[8] The ^{13}C chemical shifts assignable to C-1 through C-9a of **11–13**[4] also agree well with those of known β-carbolines.[10]

The three coupled methylene groups of **11–13** near 4.2, 2.1, and 3.3 ppm (Table II) may be assigned to H-5′, H-4′, H-3′, and ^{13}C signals near 62.0, 34.8, and 21.7 ppm [4] to C-5′, C-4′, and C-3′, respectively. The three-carbon unit $CH_2CH_2CH_2$ must be attached to the imine nitrogen at one end (CH_2 near 4.2 and 62.0 ppm) and to the C=N group (C-2′) at the other [CHNAc of **15** (Table II) coupled ($J_{2',3'}$ = 6.7 Hz) to a terminal CH_2 group (near 3.3 and 21.7 ppm)], thus completing the assignments as **11–13**.

Two additional eudistomins belong to this 1-pyrrolinyl-β-carboline ring system. More polar, eudistomins P [**16**, mp 128–130 °C ($C_{15}H_{13}BrN_3O$, HRFABMS Δ 1.6 mmu)] and Q [**17**, mp 120–125 °C ($C_{15}H_{14}N_3O$, HRFABMS Δ 0.3 mmu)] were isolated as minor products from the chloroform layer which yielded eudistomins A, D, J, M, N, and O (cf. above) and C and E.[3] Their bromohydroxy-β-carboline ring system is assigned from their UV spectra (like eudistomins D and J), while their ^1H NMR spectra (Table II) assign benzene ring patterns like those of J (P) and M (Q) and their 1-pyrrolinyl and pyridine ring pattern like that of **11–13**.

The eudistomins in the present report are all considered to be biosynthetically derived from 1 mol of tryptophan (C-3–C-9a, N-2, N-9). Eudistomins A and M, as well as G, H, I, P, and Q, are presumed to contain, in addition, glutamate-derived units—C-1 and the pyrrole ring in A and M, C-1, and the pyrrolinyl ring in G, H, I, P, and Q.

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Registry No. 1, 88704-36-3; **2**, 88704-37-4; **3**, 88704-38-5; **4**, 88704-39-6; **5**, 59444-69-8; **6**, 88704-40-9; **7**, 88729-60-6; **8**, 88704-41-0; **9**, 88704-42-1; **10**, 88729-61-7; **11**, 88704-43-2; **12**, 88704-44-3; **13**, 88704-45-4; **14**, 88704-46-5; **15**, 88704-47-6; **16**, 88704-48-7; **17**, 88704-49-8.

Supplementary Material Available: UV data for eudistomins and their derivatives and ^{13}C NMR shifts of **1** and **11–13** (2 pages). Ordering information is given on any current masthead page.

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cis-Diamminedichloroplatinum(II) Induced Distortion in a Double-Helical DNA Fragment

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Since Rosenberg's discovery,[2] that *cis*-diamminedichloroplatinum(II) (cis-platinum or cis-Pt) displays antitumor activity, findings from several laboratories clearly indicate that the bifunctional cis-Pt reacts with DNA after hydrolysis inside the cells, resulting in *cis*-$Pt(NH_3)_2^{2+}$ binding preferentially to two neighboring guanine bases on the same strand of DNA.[3] This suggestion was originally made by Stone, Sinex, and Kelman[3a] and subsequently evidenced by Bauer, Lippard, Haseltine, and coworkers.[3b-d] Several authors have suggested that the thus induced double-helix distortion is quite severe, resulting in denaturation of the DNA up to several base pairs.[4] In order to study this proposal, we investigated the decamer double helix (III) (see abbreviations)[5] after binding of cis-Pt to the central G-G sequence.

Our results indicate that—at least below 28°C—all central base pairs remain intact after chelation of *cis*-$Pt(NH_3)_2^{2+}$ by the G-G sequence. However, structural changes are induced, and the melting temperature appears to be lowered with respect to the non-platinated duplex.

The deoxynucleotide decamers I and II were synthesised by using an improved phosphotriester approach.[6] Strand I, d(T-C-T-C-G-G-T-C-T-C), has the chelating G-G dimer situated in the center and no other reactive sites are present for Pt binding. The other strand has the complementary sequence d(G-A-G-A-C-C-G-A-G-A) (for numbering used, see abbreviations).[5]

The chelation of cis-Pt at both guanine N7 positions of the purified product, obtained after reaction of strand I with an equimolar amount of cis-Pt (I-Pt), was ascertained with the use of high-frequency proton NMR. We studied the pH dependency of the nonexchangeable base protons[7] (see Figure 1), and by the

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(5) Abbreviations: cis-Pt, *cis*-diamminedichloroplatinum(II); NOE, nuclear Overhauser enhancement; DSS, 4,4-dimethyl-4-silapentanesulfonic acid sodium salt. Decamers: I, d(T-C-T-C-G-G-T-C-T-C) (numbering, T(1), C(2)–C(10)); I-Pt, d(T-C-T-C-G-G-T-C-T-C)-cis-Pt (platinum bound at both guanine N7 atoms); II, d(G-A-G-A-C-C-G-A-G-A) (numbering, G(11), A(12)–A(20)); III, I + II; III-Pt, I-Pt + II.
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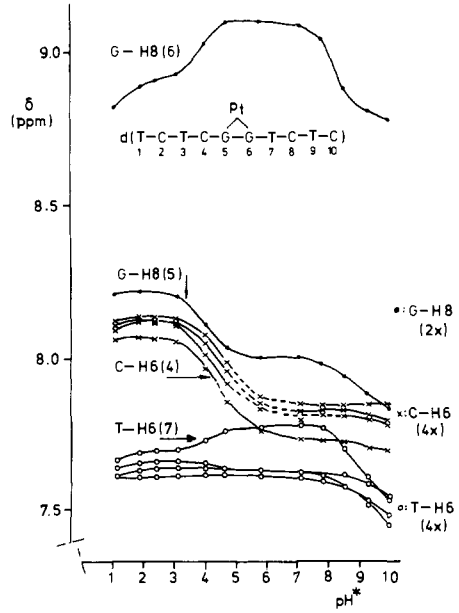

Figure 1. Chemical shift (δ) vs. pH* (uncorrected meter readings) of nonexchangeable base protons of d(T-C-T-C-G-G-T-C-T-C)·cis-Pt in D_2O (guanine H8, ●; cytosine H6, X; thymine H6, O). Chemical shifts are reported relative to DSS. NMR spectra from a 1 mM sample were obtained at 300 MHz on a Bruker WM-300 NMR spectrometer. Synthesis of the investigated compound: 5 mg of strand I was reacted with an equimolar amount of cis-Pt during 1 week in the dark (0.05 mM/L solution, pH 6, 20 °C). The compound was purified on a DEAE Sephadex A25 column (eluens, 0.0–0.7 M/L NaCl in doubly distilled water). Desalting was performed by Sephadex G25 gel filtration (eluens, doubly distilled water).

lack of downfield shifting of the H8 protons of the guanine residues near pH 2, it can be concluded that the N7 atoms are no longer accessible for protonation because of chelation, as they are in the free decamer (not shown).[8] Protonation of all cytosine residues is observed near pH 4.5, so platinum cannot be attached there. The protonation pattern closely resembles other d(G-G)·cis-Pt chelates in tetra-[9] and hexanucleotides,[10] and assignments of protons with their coupling constants leads to the conclusion that the structure of the G(5)-G(6)·cis-Pt chelate resembles that of d(G-G)·cis-Pt.[10]

In the second part of the investigation we added an equimolar amount of strand II to the adduct I-Pt to yield III-Pt. The resonances of the imino protons at low field (11–15 ppm from DSS) were compared to those displayed by the non-platinated duplex III. The imino protons (guanine N1–H and thymine N3–H) can be observed in H_2O when they are involved in Watson–Crick base pairing[11] (with cytosine N3 and adenine N1, respectively). In Figure 2a the NMR spectra of the normal duplex III and of the platinated duplex III-Pt, recorded at various temperatures, are shown. The resonances were assigned on the basis of the melting behavior and detailed NOE experiments; the assignments at −4 °C are summarized in Figure 2b.

The spectra of III (Figure 2a, left) show that even at low temperatures base pair T·A(1–20) cannot be detected. Raising

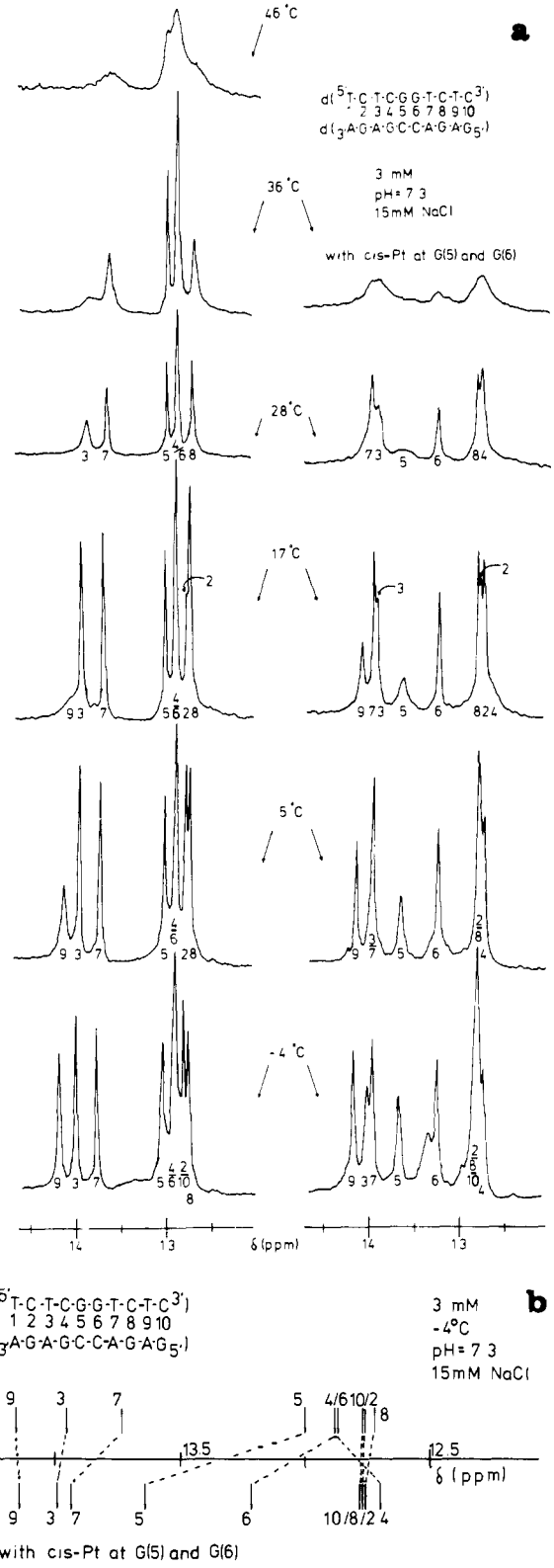

Figure 2. (a) Low-field region of the 500-MHz ^1H NMR spectra of III (left) and of III-Pt (right). Chemical shifts are reported relative to DSS. A shorthand base pair notation is used in this figure (as depicted in the right upper corner). The unassigned peak at 13.4 ppm in the −4 °C spectrum of III-Pt originates probably from base pair T·A(1–20). NMR samples were prepared by dissolving the appropiate amount of decamers in H_2O/D_2O 93/7 v/v to 3 mM/L samples (pH 7.3, 15 mM/L NaCl). Spectra were recorded on a Bruker WM-500, using a time-shared long pulse in combination with the DSA (data shift accumulation) technique in order to reduce the water signal.[13] NOE measurements (not shown) were also performed as described in ref 13. (b) Schematic representation of chemical shifts changes of imino protons upon platinum binding. Top trace, III; bottom trace, III-Pt. Chemical shifts were measured at −4 °C.

(8) The pH effect near pH 3–5 for G-H8(5) and G-H8(6) is ascribed to the protonation of the nearby cytidine; the effect near pH 8–10 for T-H6(7) is assumed to result from the deprotonation at G-N1(6). The difference in chemical shift observed for G-H8(5) and G-H8(6) is similar to earlier observations in Pt-GG chelates and originates from the conformation.

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the temperature results in "fraying", i.e., disappearing of base pairs signals for C·G(10–11) and T·A(9–12), followed by C·G(2–19) and T·A(3–18). Between 42 and 54 °C the signals of the central core (base pairs C·G(4–17) to C·G(8–13)) broaden and disappear. It can be seen (Figure 2a, right) that a lowering of T_m of III-Pt in comparison with III is apparent; the signals of the central core disappear at approximately 42 °C.[12] Surprisingly, however, at low temperature, base pairing is observed *even for the central two G·C base pairs*. The different positions for the imino protons of base pairs C·G(4–17), G·C(5–16), G·C(6–15), and T·A(7–14) in III-Pt compared to III (see Figure 2b) indicate that platinum binding has caused a change in their chemical environment.

Molecular models of a double helix, containing a d(G-G)·cis-Pt part in which the structure of the chelating part was based upon a detailed conformational analysis,[10] clearly indicates that the central two G·C base pairs can be maintained in the duplex after platinum binding but that the vertical stacking interactions between successive base pairs C·G(4–17), G·C(5–16), G·C(6–15), and T·A(7–14) are distorted. Due to platinum binding the guanine bases of G(5) and G(6) cannot maintain a parallel orientation;[10] this implicates a loss of stacking interaction between successive base pairs, which is reflected in the large downfield shifts of the imino protons of G(5) and G(6) in III-Pt compared to III. In addition, base pair T·A(7–14) is deshielded in comparison with III. This deshielding is ascribed to the loss of next-neighboring shielding of G(5). In contrast, C·G(4–17) is shielded in III-Pt with respect to III (Figure 2), indicating a specific interaction between C(4) and G(5).

It is highly surprising that, at low temperatures, a double helix can still occur after platination. Up to now, significant distortions, at 37 °C, have been deduced from other observations[4] on DNA.

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(12) UV melting profiles of III, recorded at 5×10^{-6} M/L (0.8 OD), indicate that at this concentration the melting temperature is 29 °C, concomitant with a ΔH for duplex formation of about 67 kcal/mol. UV melting profiles of III-Pt, recorded under comparable conditions, show a lowering of T_m to 14 °C. A melting profile taken at higher concentration (8.10^{-4} M/L) leads to an estimate of ΔH of 44 kcal/mol.

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Redox Reactions of a Tetrahydro-/Hexahydropyrido[2,3-*d*:6,5-*d*']dipyrimidine Tetrone Couple. A High vs. Low Potential 5-Carba-5-deazaflavin Mimic

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We have recently[1] reported details of the chemistry of 3,7,10-trimethyl-(3*H*,7*H*,9*H*,10*H*)-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetrone (PPT_{ox}) and its 1,5-dihydro reduction product

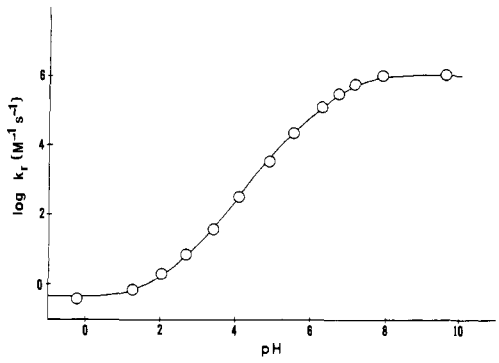
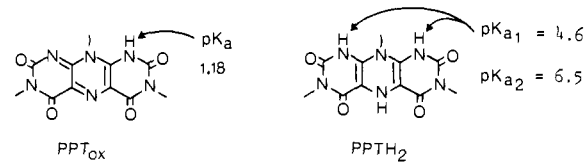
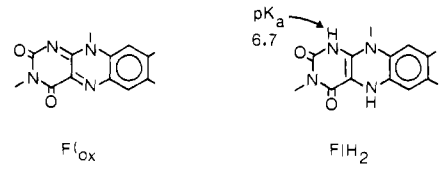


Figure 1. Plot of the log of the second-order rate constants (k_r) for the reduction of *N*-methylacridinium ion by $PPTH_{2T}$ (=$PPTH_2$ + $PPTH^-$ + PPT^{2-}) vs. pH. Points are experimental and the line is generated from eq 2 by use of the constants provided in the text (solvent H_2O, μ = 1, 30 °C).



($PPTH_2$). In the structures PPT_{ox} and $PPTH_2$, the dimethylbenzo moiety of flavins (Fl_{ox}) and 1,5-dihydroflavins (FlH_2) has been



replaced by uracil. Because of the low pK_a of PPT_{ox} (due to the extensive delocalization of the negative charge of PPT_{ox}^-) and the two enamine functions of $PPTH_2$, the redox potential (E_o' −346 mV) for the $PPTH_2/PPT_{ox}$ couple is 150 mV more negative than that for the related 3-methyllumiflavin/1,5-dihydro-3-methyllumiflavin couple. In many reactions PPT^{2-} behaves as a low potential FlH^- mimic readily reducing such substances as organic disulfides, nicotinamides, and conjugated C–C double bonds. Various aspects of the mechanism for these reactions are intriguing and remain topics of continuing investigation in this laboratory. (As an example, *m*-hydroxybenzaldehyde is reduced by PPT^{2-} to *m*-cresol without the intervention of *m*-hydroxybenzyl alcohol as an intermediate.)[1c]

5-Carba-5-deazaflavins (dFl_{ox} and $dFlH_2$) have served well as isosteric replacements for FMN and FAD in the investigation of various aspects of flavoenzyme chemistry.[2] We report herein our preliminary investigations of $dPPT_{ox}$ and $dPPTH_2$. By analogy to $PPTH_2$ one might anticipate that $dPPTH_2$ would behave as a low-potential $dFlH_2$ mimic. Further, since $E^{\circ\prime}$ for the $dFl_{ox}/dFlH_2$ couple is more negative than that for the Fl_{ox}/FlH_2 couple by 120 mV,[3] $dPPTH_2$ would appear to be a good candidate for

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