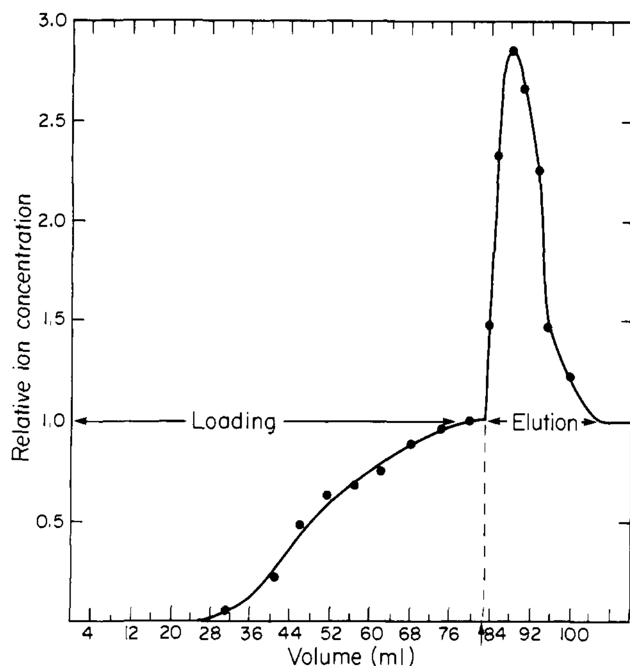

Figure 2. Spontaneous ion elution from polymeric crown ether-6 (N-K-41) by thermal shock at 60 °C in CH_3OH.

In conclusion, a polymeric crown ether system was found in which ion complexation is completely reversible and temperature dependent. This phenomenon should be of considerable interest in (i) water desalination processes by polymeric crown ethers as membranes, (ii) temperature effects in nucleophilic displacement reactions in phase-transfer catalysis by polymer-bound activated anions (e.g., [P]–CE·NaCN), and (iii) thermoregulated polymeric delivery systems for Na/K.

Registry No. (Chloromethyl)styrene divinylbenzene copolymer, 9036-15-1; catechol, 120-80-9; triethyleneglycol dichloride, 112-26-5; tetraethyleneglycol dichloride, 638-56-2; pentaethylene glycol dichloride, 5197-65-9; heptaethyleneglycol dichloride, 56930-39-3; K^+, 24203-36-9; Cs^+, 18459-37-5; Na^+, 17341-25-2; Li^+, 17341-24-1.

Intrastrand Cross-Linking of the Guanines of the Deoxytrinucleotide d(G-C-G) via *cis*-$Pt(NH_3)_2Cl_2$

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Binding of the antitumor drug *cis*-$Pt(NH_3)_2Cl_2$ (*cis*-Pt) to DNA is supposed to be a main event in its mechanism of action.[1] The kinetically most favored binding sites are the N7 atoms of the guanine bases.[2] Much evidence is accumulating that a bifunctional binding of *cis*-Pt between bases of the same strand is the predominant lesion. Generally, binding between adjacent guanine bases is considered most likely,[3] and there is evidence that such a binding in di- and tetranucleotides is possible.[4,5] However, alternative binding possibilities have been suggested, including intrastrand binding to two guanines separated by one or more other bases.[6,7] Particularly, a recent genetic study to determine the base-pair substitutions caused by *cis*-Pt in the *lacI* gene of *E. Coli* mutants showed that the majority of the substituted bases were originally part of a GAG or GCG nucleotide sequence.[7] To determine whether a cross-link between the two guanines of such a sequence is possible we have studied the interaction between *cis*-Pt and the deoxytrinucleotide d(G-C-G).

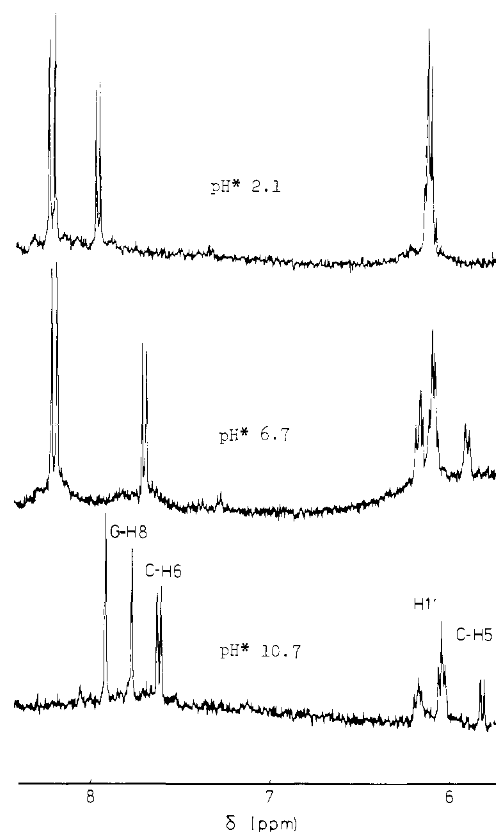

Figure 1. Part of the 360-MHz ^1H NMR spectra (8.5–5.5 ppm) of d(G-C-G)·*cis*-Pt at various pH* (pH* denotes uncorrected meter readings of solutions in D_2O). Due to the applied method for reduction of the residual HDO resonance,[13] the relative intensities of the resonances around 6 ppm are slightly smaller than those of the resonances around 8 ppm.

cis-Pt was allowed to react with 1 equiv of d(G-C-G) (Na^+ form) at room temperature for 2 weeks (concentration 10^{-5} M; pH between 6 and 7). The main product from Sephadex G-25 and Sephadex G-10 gel chromatography (accounting for approximately 90% of the total optical density at 260 nm) was isolated. Comparison of the Sephadex G-25 chromatograms of free d(G-C-G) and of its platinum adduct shows that the latter is monomeric. Platinum analysis agreed with the presence of one platinum per trinucleotide.[8] ^1H NMR spectra show that the isolated product (d(G-C-G)·*cis*-Pt) is almost pure (Figure 1). Because both cytosine N3 and guanine N7 are reported to be binding sites for platinum,[4,9] the actual binding sites were de-

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(8) A solution containing d(G-C-G)·*cis*-Pt was analyzed for Pt by flameless atomic absorption spectroscopy. From comparison with the UV absorption at λ_{max} (260 nm), an ϵ_{max} of about 24 000/mol of platinum is calculated. This value agrees with the presence of one *cis*-Pt per trinucleotide, taking into account a decrease of ϵ_{max} of about 10–20% upon binding of *cis*-Pt.[4,10]

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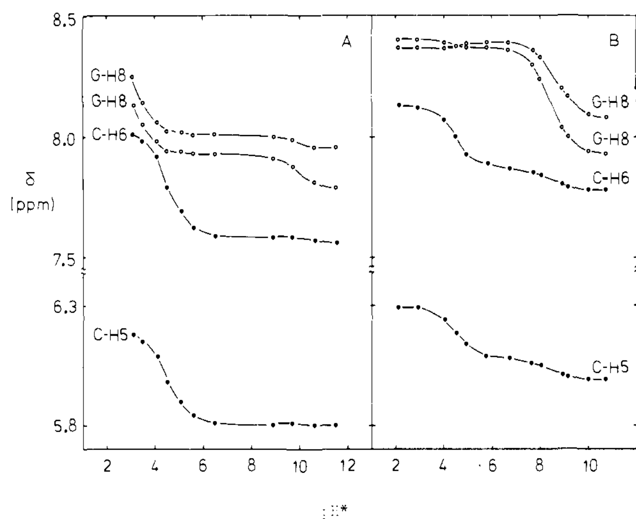

Figure 2. Chemical shift (δ) vs. pH* of the nonexchangeable base protons of the guanine (–○–) and cytosine (–●–) residues of d(G-C-G) (A) and d(G-C-G)·*cis*-Pt (B). Spectra were obtained from 2 mM solutions in D_2O at 25 ± 1 °C; tetramethylammonium nitrate was used as an internal reference (δ 3.18).

termined by a detailed analysis of the pH dependence of the chemical shifts of the base protons of d(G-C-G)·*cis*-Pt. For comparison, the pH dependence of the chemical shifts of the base protons of free d(G-C-G) was also determined (Figure 2). It is seen that all resonances of the base protons shift to lower magnetic field upon binding of *cis*-Pt. The sigmoid curves obtained upon plotting the chemical shifts of the base protons against pH* (Figure 2) can be ascribed to protonations or deprotonations of the heterocyclic nitrogens of the purine and pyrimidine bases.[4,5] The change in chemical shift for protons of free d(G-C-G) at pH 10.0 is ascribed to deprotonation of the N1 of both guanines and the chemical shift change at pH* 4.5 to protonation of the cytosine N3.[10,11] At low pH* the guanine H8 resonances start to shift strongly to lower field. This is attributed to protonation of the guanine N7 atoms, which is known to occur at about pH* 2.3.[11] So that depurination of d(G-C-G) could be avoided no spectra of this compound were recorded below pH* 3.

In d(G-C-G)·*cis*-Pt there are only two chemical shift changes with pH*: one at pH* 4.5 due to protonation of the cytosine N3 and one at pH* 8.5, which is ascribed to deprotonation of the guanine N1 atoms. Upon lowering the pH* from 3 to 2, no further chemical shift change is observed for any proton of d(G-C-G)·*cis*-Pt, indicating that protonation of the guanine N7 atoms does not occur. Undoubtedly, this is due to the fact that both guanine N7 atoms are coordinated to platinum. From the chemical shift changes at pH* 4.5 it is clear that the cytosine N3 atom is not bound to *cis*-Pt. The apparent pK_a for the deprotonation of the guanine N1 (8.5) is significantly lower than in the free trinucleotide (10.0). Such a lowering of the pK_a is common in guanine compounds with *cis*-Pt bound at their N7 atoms.[4,5,10]

Although the cytosine base is not involved in binding to *cis*-Pt, the resonances of the cytosine H5 and H6 protons are considerably shifted downfield. Chelation of *cis*-Pt by the two guanines of d(G-C-G) brings the guanines close together, so the cytosine can no longer be part of a stacked arrangement. As a result, shielding of the cytosine H5 and H6 protons by the aromatic rings of the guanine bases, which normally occurs in the stacked arrangement of the bases in oligonucleotides, will decrease, resulting in downfield shifts for these protons. The conformation of d(G-C-G)·cis-Pt could in some sense resemble a "bulged out" conformation, as proposed for purine-pyrimidine-purine sequences in ribonucleotides, where an increased stacking is observed between both purines and a decreased stacking between the purines and the pyrimidine.[12]

(10) Inagaki, K.; Kidani, Y. *J. Inorg. Biochem.* **1979**, *11*, 39.
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To assess more precisely the structural changes caused by *cis*-Pt in this trinucleotide, we are conducting a full conformational analysis of d(G-C-G)·*cis*-Pt.

Concluding, we have shown that as a result of *cis*-Pt binding, intrastrand cross-linking between two guanines separated by a third base is possible, at least at the trinucleotide level. Such a cross-link will produce a very different lesion in DNA in comparison with a cross-link via *cis*-Pt involving two adjacent guanines. This may be reflected in the mutation induction in *E. Coli* bacteria, where repair of lesions involving *cis*-Pt bound to two guanines separated by a third base apparently induces more base-pair substitutions.[7] Whether the described lesion plays a role in the mechanism of the antitumor action of *cis*-Pt remains to be investigated.

Acknowledgment. We are indebted to Professor Dr. J. H. van Boom for his kind gift of d(G-C-G) and to Dr. A. M. J. Fichtinger-Schepman for the platinum analyses. Professor Dr. R. Kaptein and K. Dijkstra are thanked for assistance at the 360-MHz (SON) NMR facility. This research was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Registry No. *cis*-Pt, 15663-27-1; d(G-C-G)Na, 81256-30-6; d(G-C-G)·*cis* Pt, 81277-04-5.

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Selectivity Profile of the Cation Radical Diels–Alder Reaction

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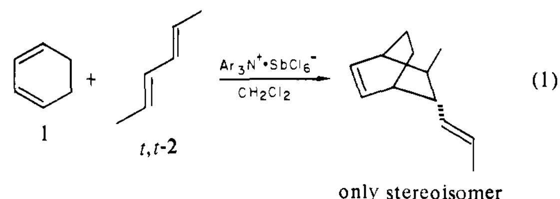
The recent observation of powerful catalysis of certain Diels–Alder cycloadditions by aminium cation radical salts[1] (the cation radical Diels–Alder, CRDA) makes conveniently available a number of Diels–Alder adduct structures that heretofore either were not directly available or were accessible in only poor to miniscule yields. In addition to the substantial intrinsic mechanistic and theoretical interest in this new reaction, it therefore appears to have potential for developing into a generally useful synthetic procedure. The suprafacial stereospecificity of the reaction, as established, for example, in the cycloaddition of 1,3-cyclohexadiene (**1**) to the three geometric isomers of 2,4-hexadiene (**2**) and illustrated in eq 1 for the trans, trans isomer, implies a



true pericyclic process analogous to the thermal (neutral) Diels–Alder. As examination of the orbital correlation diagram in Scheme I reveals, the prototype [4 + 1] cycloaddition (that between ethene cation radical and *s-cis*-1,3-butadiene to give

(1) Bellville, D. J.; Bauld, N. L. *J. Am. Chem. Soc.* **1981**, *103*, 718.

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