kcal/mol more stable than the cis isomer.[20] Thus, in the cis and trans isomers of **4** and **2**, approximately the same magnitude in the difference and the same order of strain energies seem to be present.

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(20) T. Miebach, unpublished results.

Stability of Platinated Oligonucleotide Duplexes Containing a Base Pair Mismatch at the Site Complementary to the Platination Site

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cis-Diamminedichloroplatinum(II) (cDDP) is a very clinically useful antitumor agent.[1] It is also mutagenic[2] and carcinogenic.[3] These interesting biological activities of cDDP are said to be due to preferential bifunctional chelation to two adjascent guanine residues in DNA.[4-6] This chelation induces significant decrease in the melting temperature (T_m) of the platinated oligonucleotide duplexes.[7,8] This effect is attributed to a kinked cDDP-DNA structure by NMR experiments[9] and molecular mechanics calculations.[10] Although the imino resonances of the platinated guanine residues in oligonucleotides can be observed at low temperature, these are the first to be disrupted at higher temperature[8] and there is no proof that imino resonances observed at low temperature in oligonucleotide duplexes participate in the same sort of hydrogen bonding as exists under normal conditions. Moreover, molecular mechanics calculations indicated possible unusual base pairing in platinated oligonucleotides.[10] A recent report suggests that the kinked structure does not occur in the monofunctional-platinated nonanuclotide although the double-helical structure is significantly destabilized.[11] Thus, the platination of N(7) of guanine residues in DNA may reduce their ability and selectivity for G-C base pairing. In fact, cDDP coordination at N(7) of guanines facilitates the deprotonation at N(1) of guanine ligands (pK = 8.2 compared to 9.8 for free guanine).[12,13] This causes the formation of significant amounts of both protonated and deprotonated guanine at pH 7. The deprotonated guanine forms hydrogen bonding with G or T rather than C.[14] It is still uncertain to what extent the binding of cDDP affects base pairing in an oligonucleotide.

Table I. Melting Temperatures and Hypochromicity of Four Decanucleotide Duplexes Modified and Unmodified with cDDP[a]

		T_m, °C (hypochromicity)					
		0.5 M NaCl				0.1 M NaCl	
		-Pt		+Pt		-Pt	+Pt
	X	pH 7	pH 9	pH 7	pH 9	pH 7	pH 7
duplex 1	C	54.7 (24.1%)	53.4 (23.9%)	33.5 (18.5%)	31.5 (19.2%)	49.2 (25.1%)	29.6 (19.5%)
duplex 2	T	40.6 (22.9%)	38.4 (25.2%)	11.5 (13.9%)	10.0 (13.6%)	33.5 (25.5%)	8.0 (16.1%)
duplex 3	A	37.0 (21.2%)	36.3 (22.4%)	10.0 (13.1%)	9.3 (13.6%)	30.5 (21.5%)	6.5 (13.5%)
duplex 4	G	31.4 (20.4%)	31.0 (21.2%)	N.D.[b] (8.7%)	N.D.[b] (8.9%)	23.4 (20.8%)	N.D.[b] (8.7%)

[a] T_m values were determined[20] by measuring change in absorbance at 274 nm as a function of temperature at 5.7 μM duplex concentration in 10 mM sodium phosphate buffer containing 0.5 M NaCl. When a base line at low temperature was observed, the T_m was calculated by using sloping base lines. For duplexes with T_m < 20 °C, such a base line could not be observed, and T_m values were calculated by using a flat base line. Hypochromicity is indicated in parentheses. [b] In these cases, helix-to-coil transition was not observed.

We investigated pH dependence and the effects of base substitution at the site complementary to the platination site for the stability of platinated decanuclotide duplexes so as to assess base pairing ability and the selectivity of the platinated guanine residue in oligonucleotides.

The decamers were synthesized by β-cyanoethylphosphoramidite methodology.[15] *cis*-Pt(NH$_3$)$_2$ [d(ACTCGGCTCA)-N7-G(5),-N7-G(6)] was prepared by an equimolar reaction of cDDP with d(ACTCGGCTCA). Purification was performed on a C-18 column. Finally, the product was converted to the sodium salt.[16] The synthesized decamers and Pt complex were more than 95% pure with reversed phase HPLC and identified by enzymatic digestion with nuclease P1.[17] We investigated the stability of the duplexes containing the base pair mismatches at the complementary site of the coordinated 5′-guanosine for two reasons. First, the theoretical models derived from molecular mechanics calculations suggested there was only one hydrogen bond between the 5′-guanosine and complementary cytosine on the opposite strand.[10] Second, in a preliminary study, we introduced the G-T mismatch at the site of the coordinated 3′-guanosine. The results indicated that the extent of the T_m lowering of this duplex by platination was comparable[19] with that of Duplex 2, and the T_m of Duplex 1 was decreased by this mismatch in a similar tendency

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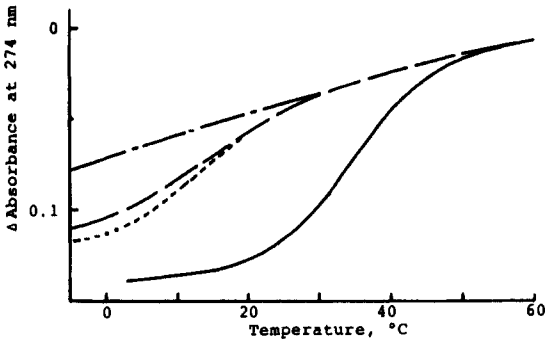

Figure 1. Melting profiles of platinated Duplexes 1-4 at 274 nm with 5.7 μM duplex concentration in 10 mM sodium phosphate buffer (pH 7) containing 0.5 M NaCl: Duplex 1 (—), Duplex 2 (---), Duplex 3 (--), Duplex 4 (---).

with the mismatch at the 5'-coordinated site with and without platination. (The T_m lowering of the platinated duplex by G-T mismatch was slightly larger than that of the nonplatinated one.)

5'-ACTCGGCTCA-3'
3'-TGAGXCGAGT-5'

X = C, T, A, G

Table I shows the T_m values of the modified and unmodified duplexes. Under a high salt condition, the decrease in the T_m of Duplex 1 by platination was 21.2 °C at pH 7,[21] which is consistent with earlier observations.[7,8] For the noncoordinated duplexes, the T_m was lowered by base substitution in the order C > T > A > G.[21] The reduction of the T_m of unplatinated Duplex 1 by the G-T mismatch was 14.1 °C and that by the G-G mismatch 23.3 °C. The destabilizing effect of the decanucleotide duplexes by platination was larger than that of the single base pair mismatches (except for the G-G mismatch), and thus the reduction of the T_m of the platinated duplexes was not due only to the alteration of the base pairing ability of the coordinated guanine residue. The extent of the T_m lowering of the platinated duplexes by base substitution was comparable with (slightly larger than) that of the unplatinated duplexes containing the corresponding base pair mismatch. The reduction of T_m by platination would not be attributable to alteration of the base pairing ability of the coordinated guanine.

Under basic conditions (pH 9), the T_m of Duplex 1 slightly decreased, regardless of platination of the duplex. The mismatched duplexes also showed the same behavior. Independent of pH, the order of stability of the platinated duplexes by the base substitution was C > T > A > G (Figure 1, Table I), the same as that of the unplatinated duplexes. Thus, effective G-T or G-G base pairing at the platination site does not occur even at pH above the pK of the platinated guanine. These G-X mismatches (X = T, A, G) showed weaker base pairing.[23] It thus appears quite likely that the base pairing selectivity of the guanine residue in the oligonucleotides is not altered by platination at the N(7) site. In addition, the results mentioned above were reproduced at more physiological conditions (Table I, 0.1 M NaCl).

It is still uncertain what decreases the stability of platinated duplexes. Table I shows the hypochromicity of each case in parentheses. At pH 7, the T_m of platinated Duplex 1 was slightly higher than that of unplatinated Duplex 4. However, the hypochromicity of the former case was even less than that of the latter case, independent of salt concentration. The base stacking disruption by platination is thus suggested and this may support the kinked cDDP-DNA structure models.[10]

In conclusion, our results suggest that cDDP coordination of N(7) of two adjascent guanines in the oligonucleotides hardly affects the base pairing ability or selectivity. The mutagenicity of cDDP may depend not on alteration of the above features of coordinated guanines but on a kinked structure or an unusual conformational feature[22] of cDDP-DNA complexes.

(21) The destabilization of the duplex structure may be attributed to stabilization of the single strand form by platination.[22] If it does so, this effect acts equally on Duplex 1-4, and therefore, the T_m of these duplexes should reflect the relative strength of the hydrogen bond of the coordinated guanines. At least the upper single strand decamer did not show the sigmoidal transition in UV vs temperature profiles, regardless of platination.

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Stannylquinones. Synthesis and Utilization as Quinone Carbanion Synthetic Equivalents

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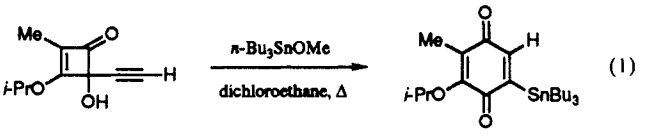
During the course of a search for effective catalysts for the metal-mediated ring expansion of 4-alkynyl-4-hydroxycyclobutenones,[2-5] an unexpected yellow-orange product was obtained in 44% yield (unoptimized) when 4-ethynyl-4-hydroxy-3-isopropoxy-2-methyl-2-cyclobuten-1-one (**1**) was treated with *n*-Bu_3SnOCH_3 in dichloroethane at reflux (eq 1). Spectroscopic analysis indicated that the alkynylcyclobutenol had been transformed into 2-isopropoxy-3-methyl-6-(tri-*n*-butylstannyl)-1,4-benzoquinone (**2**), a representative of a previously unknown class of compounds: stannylquinones.



(1)

1 **2**

The generality of this stannylquinone synthesis was established by preparation of the new compounds indicated in Table I. The precursor alkynylcyclobutenones and alkynylbenzocyclobutenones **4** were readily prepared according to established techniques[7] by addition of alkynyl anions to the substituted cyclobutenediones or benzocyclobutenediones **3**, available by previously described procedures.[8-13] Thermolysis of ≤0.1 M solutions of **4** in dichloroethane in the presence of 1.05 equiv of *n*-Bu_3SnOMe for 10-15 min led to good yields of most of the stannylquinones (2,3-dialkoxycyclobutenone and some of the benzocyclobutenone substrates took 2-4 h to react to completion); however, a few of the products were formed in low yield for reasons not yet determined. The stannylquinones, when pure, are fairly stable open

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