A double stranded DNA fragment shows a significant decrease in double-helix stability after binding of monofunctional platinum amine compounds

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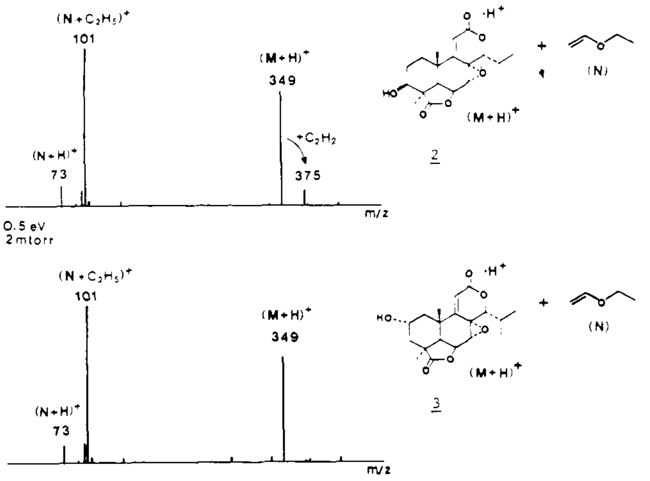
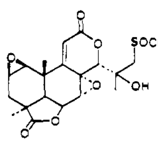


Figure 1. The product distributions obtained for two protonated, isomeric diterpenoid dilactones upon collisions with ethyl vinyl ether in the center quadrupole of a triple quadrupole mass spectrometer. The base peak (101^+) arises from ethylation of ethyl vinyl ether by protonated ethyl vinyl ether (73^+).[5c,7]

terium at a nonacidic site, as evidenced by the fact that this deuterium cannot be exchanged to a hydrogen atom upon collisions with ethyl vinyl ether. The large difference in the heat of formation of simple neutral alkyl vinyl ethers and alcohols ($\Delta(\Delta H_f)$ = −22 kcal/mol for ethyl vinyl ether and ethanol) is the most likely driving force for this entropically disfavored reaction. We estimate the reaction to be at least 4 kcal/mol exothermic for protonated 4-hydroxy-2-butanone and ethyl vinyl ether.[10] Note that the proton affinities of β-hydroxycarbonyl compounds are comparable to the proton affinities of alkyl vinyl ethers.[10] This precludes efficient competition by the reaction that usually dominates the ion chemistry of alkyl vinyl ethers, i.e., deprotonation of the reactant ions.

The most intriguing result of this study is the discovery that "vinylation" of organic ions by alkyl vinyl ethers is highly selective toward different oxygen-containing functionalities and that this behavior is not limited to simple model ions but applies to complex polyfunctional ions as well. Under the same conditions, only dissociation and deprotonation reactions were observed for a number of protonated alcohols, ethers, aldehydes, ketones, esters, lactones, and epoxides, including mono- and polyfunctional, saturated and unsaturated, cyclic and acylic as well as aromatic molecules. To test the selectivity of the "vinylation" reaction more rigorously, we decided to examine the reactivity of polyfunctional molecules that *only* differ by the position of the hydroxy group expected to be necessary for the reaction. The isomeric diterpenoid dilactones **2** and **3** (Figure 1) present a challenging test since these protonated molecules are difficult to distinguish on the basis of their dissociation product distributions. However, due to steric constraints in the isomer **3**, only **2** is expected to exchange a proton to a vinyl group upon collisions with ethyl vinyl ether. We found that protonated **2** does indeed undergo the reaction of interest, giving a product ion with a relative abundance of up to 10% of the base peak (101^+), while **3** only gives a trace at the mass value of interest (≤1% of the base peak; Figure 1). Moreover, the reaction seems to be independent of the structure of the rest of the molecule. For the six diterpenoid dilactones (**1**–**6**) shown in Scheme I, Figure 1, and below, the reaction is limited to those molecules (**1**, **2**, **6**) that contain a 3-hydroxy functionality in the A ring.



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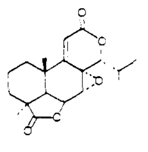

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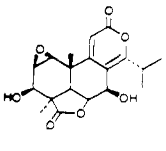

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Acknowledgment. This work was supported by the National Science Foundation (CHE-8721768, RGC; CHE 8717380, HIK). Professor J. Cassady is acknowledged for samples of **1**–**6**.

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(10) ΔH_f of protonated 4-hydroxy-2-butanone was estimated to be ≥62.9 kcal/mol by assuming that the proton affinity of 4-hydroxy-2-butanone is equal to or less that of acetyl acetone (i.e., ≤207.8 ± 5 kcal/mol[11]), and estimating ΔH_f for neutral 4-hydroxy-2-butanone (estimated to be −95.4 kcal/mol by the method of Benson[12] and using ΔH_f of 2-butanone: −57.5 kcal/mol[11]); ΔH_f of α-deprotonated 4-hydroxy-4-methylpyran was estimated to be +80.9 kcal/mol by the method of Benson[12] and using ΔH_f = +129 kcal/mol for α-deprotonated pyran;[11] ΔH_f of ethyl vinyl ether is −34 kcal/mol;[11] ΔH_f of ethanol is −56.1 kcal/mol.[11]

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A Double-Stranded DNA Fragment Shows a Significant Decrease in Double-Helix Stability After Binding of Monofunctional Platinum Amine Compounds

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The antitumor drug *cis*-diamminedichloroplatinum(II) (cDDP[1]) preferentially binds to two neighboring guanine bases of DNA.[2-4] It has been suggested that this chelation induces a serious distortion of the DNA, resulting in a denaturation up to several base pairs.[5,6] Recently, NMR studies and molecular mechanics of oligonucleotides containing eight or more base pairs showed that the distortion is small; all base pairs, even those of the platinated guanines, are observed.[7,8] Nevertheless, the melting temperature (T_m) appeared to be lowered by 10–20 °C. These phenomena have been attributed to a "kinked" cDDP-DNA structure.[9,10]

For a detailed understanding of the working mechanism of cDDP not only the ultimate structural change but also the distortion resulting from the first binding step is important.

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(1) Abbreviations: cDDP, *cis*-$PtCl_2(NH_3)_2$; tDDP, *trans*-$PtCl_2(NH_3)_2$; dien, diethylenetriamine; dsNONA, d(TCTCGTCTC)·d(GAGACGAGA); Pt(dien)-dsNONA, Pt(dien)[d(TCTCGTCTC)-$\underline{N}$7(5)]·d(GAGACGAGA); $Pt(NH_3)_3$-dsNONA, $Pt(NH_3)_3$[d(TCTCGTCTC)-$\underline{N}$7(5)]·d(GAGACGAGA); T_m, melting temperature.

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Table I. Melting Temperatures of Three Oligonucleotides Modified with cDDP and One Nonanucleotide Platinated with $[Pt(dien)]^{2+}$ or $[Pt(NH_3)_3]^{2+f}$

oligonucleotide	duplex	added salt	$T_m(-Pt)$ (°C)	$T_m(+Pt)$ (°C)	ΔT_m (°C)	ref
d(GATCC$\underline{GG}$C)·d(GCCGGATCGC)	(Pt–)A^a	1 M NaCl	55	28	27	8
d(GCC$\underline{GG}$ATCGC)·d(GCGATCCGGC)	(Pt–)B^b	1 M NaCl	58	49	9	21
d(TCTC$\underline{GG}$TCTC)·d(GAGACCGAGA)	(Pt–)C^c	none	29	14	15	7
d(TCTC$\underline{GG}$TCTC)·d(GAGACCGAGA)	(Pt–)C^d	0.1 M Mg^{2+}	52	30	22	22
d(TCTC$\underline{G}$TCTC)·d(GAGACGAGA)	*e*	0.5 M NaCl	42	26	16	this work

^{a}A, 23 μM; Pt–A, 8 μM. ^{b}1 μM. ^{c}5 μM. ^{d}6.4 μM. ^{e}4–5 μM. ^{f}The platinated sequences are underlined.

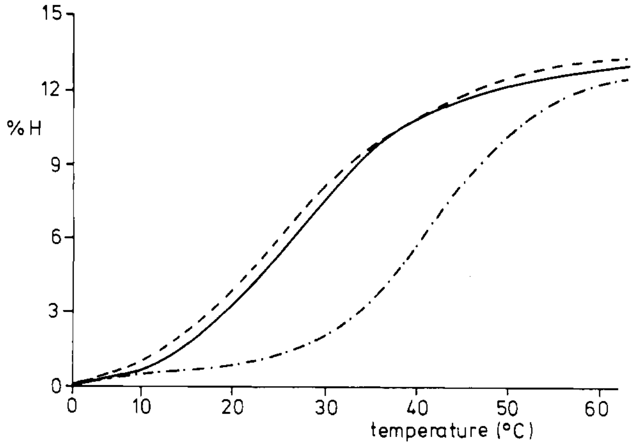

Figure 1. Melting profiles of dsNONA (–·–), Pt(dien)-dsNONA (—), $Pt(NH_3)_3$-dsNONA (––) at 260 nm, 4–5 μM nonamer strand, 0.5 M NaCl, pH 7, temperature progression 1 °C/min, %H = %hyperchroism.

Therefore, we have investigated the double-stranded nonamer d(TCTCGTCTC)·d(GAGACGAGA) modified with the monodentate complexes [PtCl(dien)]Cl and $[PtCl(NH_3)_3]Cl$, which are bound to the central guanine base.

In contrast with the results of others,[11,12] our experiments show that the T_m of the studied nonamer is significantly decreased upon monofunctional platinum binding. This is indicative for a destabilization of the double helix.

The nonamers d(TCTCGTCTC) and d(GAGACGAGA) were synthesized via an improved phosphotriester method.[13] Pt(dien)[d(TCTCGTCTC)-$\underline{N}$7(5)] and $Pt(NH_3)_3$[d(TCTCGTCTC)-$\underline{N}$7(5)] were obtained after an equimolar reaction of the platinum compound with the nonamer. The reaction products were identified by NMR. After addition of the complementary strand a duplex is formed; ^1H NMR shows that all imino protons are present, even the proton of the platinated G·C base pair (data not shown).

The melting behavior of the two Pt-containing fragments and the unmodified compound were studied. The experimental setup has been described previously.[14]

Under low salt conditions, above 2 °C, the melting profiles of the Pt nonamers could not be observed completely. Therefore, NaCl was added to stabilize the duplexes. Figure 1 shows the melting profiles of the oligonucleotides under comparable conditions. The T_m of the unmodified nonamer is 42 °C. Platination of the fragment with $[Pt(dien)]^{2+}$ or $[Pt(NH_3)_3]^{2+}$ reduces the melting temperature to 26 °C.[15]

Although several authors predicted a destabilization of the DNA duplex due to fixation of monodentate platinum,[16] such a drastic decrease in T_m has not yet been reported. Hermann et al.[16a] explained the stabilization of the poly(I)·poly(C) duplex after $[Pt(dien)]^{2+}$ binding with a triple-sandwich structure, i.e., the hypoxanthine-N7 atoms above and below the one to which Pt(dien) is bound form hydrogen bonds with protons of the two amino groups of the Pt(dien) moiety. The formation of such a triple-sandwich structure is neighbor dependent and is not likely to occur in our nonamer sequence. These authors also investigated the monofunctional interaction of *trans*-$PtCl_2(NH_3)_2$ (tDDP) with poly(I)·poly(C).[17,18] In agreement with our results, a destabilization of the duplex was found;[18] the T_m was lowered significantly after tDDP binding. The observation that [PtCl(dien)]Cl facilitates the B → Z transition in poly(dG-dC)·poly(dG-dC)[19,20] is another indication that the DNA structure can be significantly affected by monodentate platination.

For oligonucleotides (8–10 base pairs) modified with cDDP a reduction in the melting temperature of 9–27 °C has been reported[7,8,21,22] (Table I). Nevertheless, all imino protons of these duplexes are observed with ^1H NMR.

The melting temperature appears to be dependent on the number of base pairs involved in duplex formation and the location of the platination site (compare T_m and ΔT_m of duplex A and B, Table I). The base sequence of the fragment is also important. This is demonstrated by duplex B and C, which are both decamers with the platination site in the middle of the sequence. However, the reduction of the T_m of these decamers is very different, namely 9 and 15 °C, respectively.

In comparison to duplex C our nonamer lacks a guanine–cytosine base pair in the center. For this fragment we measured a T_m lowering of 16 °C due to monofunctional platinum binding, which compares quite well to a reduction of 15–22 °C due to bifunctional platination of the related C duplex.

Although the results collected in Table I were obtained under different conditions it is obvious that the reduction of the T_m due to both bifunctional and monofunctional platinum binding is in the same order of magnitude. This result is unexpected in the light of earlier observations.[23-25] Binding of [PtCl(dien)]Cl to random sequence DNA hardly influences the CD spectrum, and the change of the CD induced by bifunctional binding of cDDP is mainly due to the chelation step.[23,24] Moreover, $[PtCl(dien)]^+$ does not disrupt the duplex of calf thymus DNA in contrast to the effects of cDDP and tDDP.[25]

In conclusion, binding of a monofunctional platinum compound distorts the DNA structure significantly. The decrease in melting

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temperature appears to be almost the same for mono- and bifunctional Pt compounds. Already in the first platination step the duplex is destabilized, whereafter chelation can take place to form a "kinked" structure, apparently without further major helix destabilization. The details of the helix distortion studied with CD and NMR is the subject of future investigations.

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Elaboration of Fused *gem*-Dimethylcyclopropane Systems via Cyclopropene Cycloaddition. A Stereocomplementary Approach

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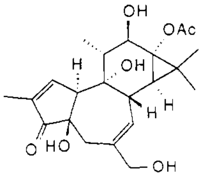
Received February 10, 1989

A *gem*-dimethylcyclopropane unit fused to a six-membered carbocycle is a commonly displayed architectural feature characteristic of natural products as structurally diverse as the tumor-promoting diterpene, phorbol (**1**),[1] and the sesquiterpene, aristolone (**2**).[2]



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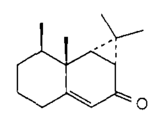

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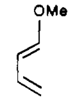
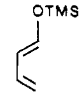
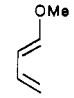
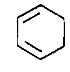
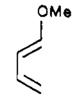
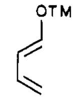
To date, access into the bicyclo[4.1.0]heptane carbon skeleton has most often employed dihalocarbene insertion–organocopper substitution technology, which often provides the target in only modest overall yields.[3,4] We wish to report a highly stereoselective, general protocol based on cyclopropene cycloaddition chemistry as an alternative method for the elaboration of fused *gem*-dimethylcyclopropane species. A noteworthy aspect of this methodology is the capability of assembling systems with complementary stereoselection by minor reaction sequence modification.

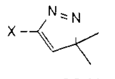
While several substituted cyclopropene species have displayed dienophilic behavior in simple systems,[5] *gem*-dimethylcyclopropene itself is a notoriously poor participant in the Diels–Alder reaction.[6] We were, however, intrigued with the notion of exploiting the cycloaddition chemistry of the carbonyl-activated dimethylcyclopropene series as an attractive entry into the CD rings of phorbol (**1**) and related diterpenes.

Table I. Cycloadditions of Cyclopropane and Pyrazole Addends with Dienes

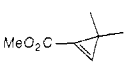
entry	diene	dienophile	pressure (kbar)	exo:endo	% yield of cyclopropane[a]
1		**3a**	10	1:22	92[b,c]
2		**3a**	10	1:5	89[b,c]
3		**3b**	10	1:19	98[b,c]
4		**3b**	10	1:50	92[b,c]
5		**4**	10	50:1	81[c]
6		**4**	10	50:1	78[c]

[a] All new compounds reported herein exhibited satisfactory spectral (IR, ^{1}H NMR, ^{13}C NMR), analytical, and/or high resolution mass spectral characteristics. [b] Overall yield for cycloaddition and quantitative photochemical nitrogen extrusion. [c] Yield of isolated, purified products.

The requisite addends **3a,b** and **4** were readily prepared by cycloaddition of 2-diazopropane to the appropriately functionalized acetylenes followed, in the case of **4**, by photochemically induced nitrogen extrusion.[7] Typically, thermal cycloaddition reactions (CH_2Cl_2, 50 °C, 96 h) of these reagents required massive excesses of diene to assure adequate yields of adducts. However, performing the additions at high pressure (CH_2Cl_2, 8–10 kbar, 18 h) provided excellent yields of products employing essentially 1:1 diene–dienophile stoichiometry. The results of the reaction of **3a** and **4** with (*E*)-1-acetoxy-1,3-butadiene are illustrative.



3a, X=CO_2Me
3b, X=COMe



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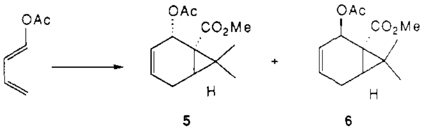

5 **6**

Exposure of this diene to cyclopropene **4** (CH_2Cl_2, 10 kbar, 18 h) provided adducts **5** (exo) and **6** (endo) in 78% yield with an exo:endo ratio of 50:1[8,9] paralleling the established proclivity for exo addition exhibited by hindered cyclopropenes.[5a] *In marked contrast, pyrazole* **3a** *gave, after cycloaddition* (CH_2Cl_2, *10 kbar, 18 h*) *and quantitative photochemical nitrogen extrusion* (*3500 Å, 2.5 h*) *from the bicyclic pyrazoline intermediate, a 93% overall yield of a mixture of* **5** *and* **6** *in which the endo diastereomer* **6** *prevailed in a ratio of 50:1.*[10] Thus, effective complementary

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(1) For synthetic studies on the phorbol system, see: Wender, P. A.; Keenan, R. M.; Lee, H. Y. *J. Am. Chem. Soc.* **1987**, *109*, 4390 and references cited therein.

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(8) The exo/endo designation is relative to the *gem*-dimethylcyclopropane moiety.

(9) The corresponding *Z*-dienes do not react to any appreciable extent under these conditions as evidenced by the total absence of reactivity of (*Z*)-1-acetoxy-1,3-butadiene toward **3a** and **4**.

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