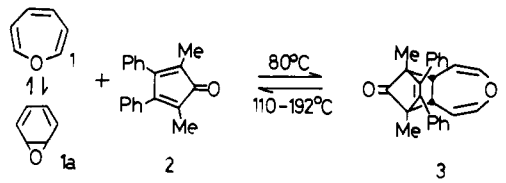
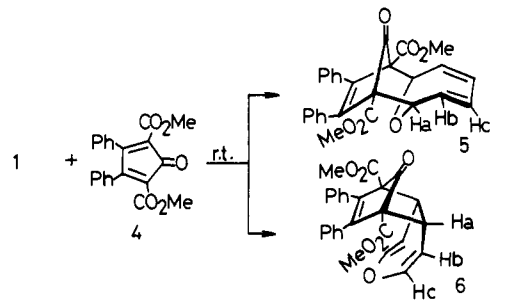
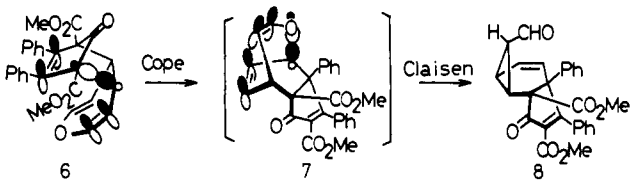
Scheme I



Scheme II



Scheme III



However, a striking contrast for thermal stability between **5** and **6** is observed: **5** is very stable during prolonged exposure to boiling benzene. On the other hand, **6** was converted to **8** (mp 241-244 °C) in near quantitative yield upon refluxing in benzene for 3 days. Compound **8** showed a positive response to Tollens reagent (a solution of silver ammonium hydroxide and sodium hydroxide), suggesting the presence of an aldehyde group. In fact, the IR spectrum showed three types of carbonyl bands at 1700 (enone), 1710 (aldehyde), and 1745 (ester) cm^{-1}. The NMR spectrum exhibited characteristic signals of the aldehyde proton at δ 9.45 (1 H, d, J = 7.1 Hz), the cyclopropane ring protons at δ 1.26 (1 H, ddd, J = 8.6, 8.6, 3.7 Hz), δ 1.70 (1 H, ddd, J = 8.6, 8.6, 7.1 Hz), and δ 2.29 (1 H, t, J = 8.6 Hz), and the olefinic protons at δ 5.39 (1 H, dd, J = 10.5, 3.7 Hz) and δ 5.55 (1 H, d, J = 10.5 Hz). The ^{13}C NMR spectrum showed well-resolved patterns (seven detectable sp^3 carbons at 18.34, 24.49, 33.93, 51.97, 52.32, 57.36, and 62.81 ppm). From these data, the structure of **8** was determined as a rearrangement product, as depicted in Scheme III.

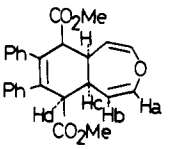
The results indicate that the initial Cope rearrangement of **6** is more significant because the effective orbital interactions in three systems activate groups among the HOMOs of the two π bonds (the vinyl ether and diphenylethylene moieties) and the LUMO of the σ bond adjacent to the electron-attracting ester group. This is in sharp contrast to Anastassiou's conclusion for the adduct **3**.[2] Furthermore, it is apparent that successive Cope rearrangements (Claisen rearrangements) lead to the highly strained compound **8**.

From these results, it is noted that the success of **1** in functioning as a 6π donor toward **4** could conceivably be caused by the frontier-control and dominant donor-acceptor interaction.[3] The powerful electron-attracting **4**[4] should be more readily trapped by electron-donating oxepin, *even in the existence of the valence-tautomeric equilibrium between benzene oxide and the nonplanar conformational isomers.*[5]

(3) Inagaki, S.; Fujimoto, H.; Fukui, K. *J. Am. Chem. Soc.* **1976**, *98*, 4693-4701.

(4) It is interesting that the CNDO/2 MO calculation indicates a 0.7-eV lowering of the LUMO energy for **4** as compared to **2**: Yasuda, M.; Harano, K.; Kanematsu, K. *Tetrahedron Lett.* **1980**, 627-630.

Finally, treatment of the adduct **6** with silicic acid in a protic solvent gave compound **9** [mp 185-187 °C, $C_{26}H_{24}O_5$, mass



9

spectra, m/e 416 (M^+)] in a moderate yield. The absence of the bridged carbonyl band in the IR, three detectable sp^3 carbons (39.44, 50.77, and 51.89 ppm) in the ^{13}C NMR, and characteristic signals at δ 3.17 (2 H, H_c, m), δ 3.18 (6 H, s, $CO_2Me \times 2$), δ 3.66 (2 H, H_d, d, J = 6.8 Hz), δ 5.17 (2 H, H_b, dd, J = 7.2, 6.2 Hz), and δ 6.22 (2 H, H_a, dd, J = 7.2, 1.3 Hz) in the NMR suggested the structure of **9** resulting from decarbonylation followed by hydrogen abstraction from the polar solvent, although further studies are necessary to settle the formation mechanism.

In addition, qualitative similarities in reactivity between two dienes are expected from the calculated FMO energy levels of **2** and tetracyclone,[4] but there is a large difference in reactivity between the reactants: tetracyclone reacts with **1** much slower (for about 1 month) than with **2** or **4**, even under more drastic conditions. We are currently examining the cycloadditivity and periselectivity of **1** with other cyclopentadienones on the basis of the frontier-controlled donor-acceptor interaction theory.[3]

(5) (a) Especially noteworthy is the fact that the absence of valence tautomerism of the 1*H*-azepine-azanorcaradiene form is contrasted with those for oxepin; see ref 1. (b) After the work had been submitted for publication, study of the benzene oxide-oxepin valence isomerization was reported: Hayes, D. M.; Nelson, S. D.; Garland, W. A.; Kollman, P. A. *J. Am. Chem. Soc.* **1980**, *102*, 1255-1262.

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Chirality at a Pro-pro-prochiral Phosphorus Center. Stereochemical Course of the 5'-Nucleotidase-Catalyzed Reaction

Sir:

We report the first study on a stereochemical problem involving a pro-pro-prochiral phosphorus center,[1] the hydrolysis of AMP[2] to adenosine and P_i catalyzed by the venom 5'-nucleotidase, by use of chiral [^{16}O,^{17}O,^{18}O]thiophosphates (Ps_i).

Scheme I summarizes our experimental approaches. Reaction of $PSCl_3$ with adenosine,[3] followed by $H_2{}^{18}O$ (99%) hydrolysis, gave [$^{18}O_2$]AMPS (**1**) (>98% ^{18}O). Chemical phosphorylation[4] of **1** yielded [α-$^{18}O_1$]ADPαS (A + B) (**2**). Incubation of **2** with pyruvate kinase[5] gave [α-$^{18}O_1$]ATPαS (A) (**3**) and unreacted [α-$^{18}O_1$]ADPαS (B) (**4**).[6] Reaction of **3** and **4** with alkaline

(1) Problems involving a chiral, prochiral, or pro-prochiral ($ROPO_3^{2-}$) phosphorus center have been solved recently: Knowles, J. R. *Annu. Rev. Biochem.*, in press.

(2) Abbreviations used: P_i, inorganic phosphate; Ps_i, inorganic thiophosphate; O, oxygen-16; ⊖, oxygen-17; ●, oxygen-18; AMP, adenosine 5'-monophosphate; AMPS, adenosine 5'-thiophosphate; ADPαS, adenosine 5'-(1-thiodiphosphate); ATPαS, adenosine 5'-(1-thiotriphosphate); ADPβS, adenosine 5'-(2-thiodiphosphate); ATPβS, adenosine 5'-(2-thiotriphosphate); ATPγS, adenosine 5'-(3-thiotriphosphate). The diastereomers A and B are designated on the basis of their enzymatic activity (ref 4).

(3) Murray, W. A.; Atkinson, M. R. *Biochemistry* **1968**, *7*, 4023-4029.

(4) Eckstein, F.; Goody, R. S. *Biochemistry* **1976**, *15*, 1685-1691.

(5) (a) Richard, J. P.; Ho, H. -T.; Frey, P. A. *J. Am. Chem. Soc.* **1978**, *100*, 7756-7757. (b) Jaffe, E. K.; Cohn, M. *J. Biol. Chem.* **1979**, *254*, 10839-10845.

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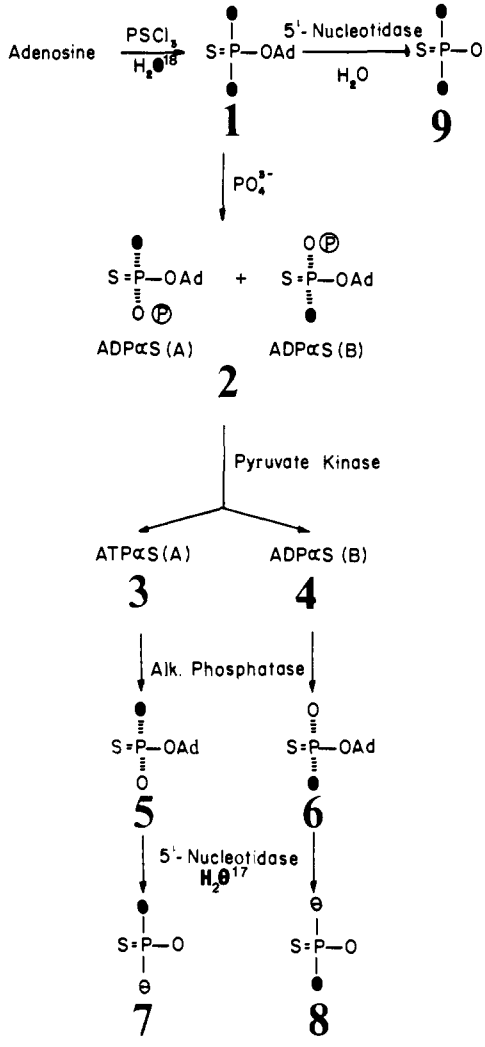
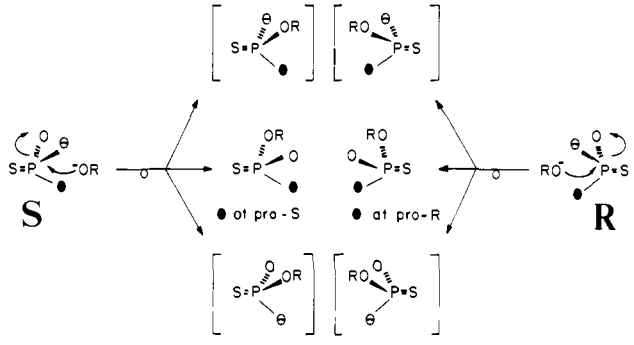
Scheme I



Scheme II



phosphatase gave (S_p)-[$^{18}O_1$]AMPS (**5**) and (R_p)-[$^{18}O_1$]AMPS (**6**), respectively (>95% ^{18}O). Hydrolysis of **5** and **6** by the venom 5′-nucleotidase in $H_2{}^{17}O$ (52.8% ^{17}O, 41.8% ^{18}O) gave chiral [^{16}O,^{17}O,^{18}O]Ps_i **7** and **8**, respectively, of unknown configuration. In a separate set of experiments, **1** (85% ^{18}O) was hydrolyzed in the presence of 5′-nucleotidase in H_2O to give $PS^{18}O_2O^{3-}$ (**9**) which was enriched with only 65 atom % ^{18}O at both labeled positions. Thus, approximately 1.5 oxygen atoms from solvent were incorporated into the product Ps_i.[7]

(6) Pyruvate kinase is not 100% specific for isomer A. However, the product from the first 35% reaction was >95% pure ATPαS (A) whereas the last 35% unreacted ADPαS was >95% pure ADPαS (B), as determined by NMR analysis.

(7) This is mechanistically significant since one and only one oxygen atom is incorporated into P_i in the hydrolysis of AMP: Koshland, D. E., Jr.; Springhorn, S. S. *J. Biol. Chem.* **1956**, *221*, 469–476.

Scheme III

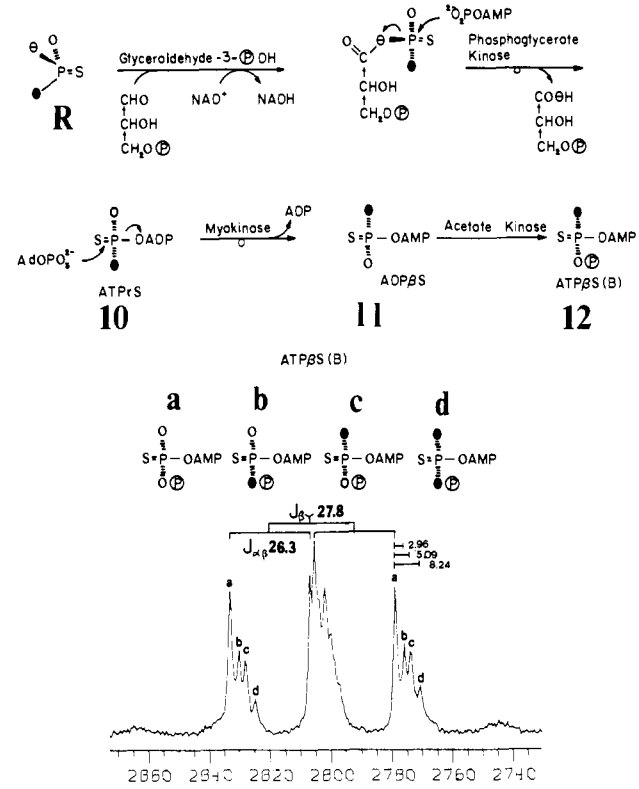


Figure 1. Pβ signal of the ^{31}P NMR spectrum of the ATPβS (B) obtained from $PS^{18}O_3{}^{3-}$ (40 atom % ^{18}O). The sample (40 μmol) was dissolved in 2.5 mL of D_2O containing 10 mM EDTA, and the spectrum was recorded at 145.7 MHz. The unit is expressed in hertz. The upfield shifts of signals b, c, and d are 0.0203, 0.0349, and 0.0565 ppm, respectively.

Configurational analysis of **7** and **8** is based on the combination of the $^{31}P(^{17}O)$[8] and the $^{31}P(^{18}O)$[9] NMR methods. As illustrated by Scheme II, displacement of one of the three oxygen isotopes of (S)-[^{16}O,^{17}O,^{18}O]Ps_i by a nucleophile (RO^-) gives a mixture of three unseparable species. Among them, two (those in brackets) contain an ^{17}O atom which quenches their ^{31}P NMR signals by its quadrupolar effect.[8] Only the species which contains ^{16}O and ^{18}O (^{18}O at the *pro-S* position) will show an observable ^{31}P signal. The (R)-Ps_i should give a corresponding species with ^{18}O at the *pro-R* position.

Experimentally, $PS^{18}O_3{}^{3-}$ (40% ^{18}O) and the two chiral Ps_i **7** and **8** were converted at ATPγS (**10**) (55% yield) by the combined action of D-glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase (PGK).[10] The γ-thiophosphoryl group of **10** was transferred to AMP to give ADPβS (**11**) by myokinase (inversion of configuration)[11] (80% yield). Scheme III illustrates this process with (R)-Ps_i by showing only the species which will give an unquenched ^{31}P NMR signal. Whether ^{18}O is located at the *pro-R* or *pro-S* position of **11** can be determined by stereospecific phosphorylation of **11** at the *pro-R* oxygen by acetate kinase to give ATPβS (B) (**12**).[5a] A nonbridge ^{18}O should cause a larger upfield shift of the ^{31}P NMR signal than a bridge ^{18}O does, due to a greater double-bond character.[12]

Figure 1 shows the Pβ signal of the ATPβS (B) obtained from $PS^{18}O_3{}^{3-}$. The signal contains two overlapping doublets due to ^{31}P–^{31}P coupling. Each half of a doublet contains four lines due to the four species a, b, c, and d. We define the ratio b/c as the

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(12) (a) Cohn, M.; Hu, A. *J. Am. Chem. Soc.* **1980**, *102*, 913–916. (b) Lowe, G.; Potter, B. V. L.; Sproat, B. S.; Hull, W. E. *J. Chem. Soc., Chem. Commun.* **1979**, 733–735.

Table I. ^{31}P NMR Analysis of the ATPβS (B) Derived from Chiral Thiophosphates

Ps_i samples	% intensity[a] a	b	c	d	"F" value	configuration
$PS^{18}O_3^{3-}$	41.3 ± 1.2	24.6 ± 0.1	22.1 ± 0.0	11.8 ± 1.2	1.11	
7	8.8 ± 0.5	42.8 ± 0.6	28.1 ± 0.5	20.3 ± 0.5	1.52	*S*
8	12.2 ± 0.5	26.5 ± 1.6	38.8 ± 0.1	22.4 ± 2.0	0.68	*R*

[a] Obtained from peak-height measurements for the Pβ signal of ATPβS. The errors represent deviations between the two nonoverlapping halves of the two doublets.

"*F*" value. The relative intensities are dependent on isotopic enrichments. However, any nonchirally labeled Ps_i should contribute equally to b and c to give $F = 1.0 \pm 0.1$ (reproducibility of peak heights is ±10%).

Table I lists the relative heights of the peaks corresponding to a–d from various Ps_i samples. While this work was in progress, Webb and Trentham had determined that the phosphoryl transfer catalyzed by PGK proceeds with inversion of configuration[13] by use of a similar NMR analysis. On the basis of this finding, (*R*)-Ps_i should give rise to c ($F < 1$) whereas (*S*)-Ps_i should give rise to b ($F > 1$). Since **7** and **8** gave *F* values of 1.52 and 0.68, respectively, the configurations of **7** and **8** are "*S*" and "*R*", respectively. These results indicate that hydrolysis of AMPS by 5′-nucleotidase proceeds with inversion of configuration. Thus, unlike alkaline phosphatase,[14] the reaction of 5′-nucleotidase seems to be a single displacement without involving a phosphorylenzyme intermediate.

On the basis of the enrichments of the isotopes used, the optimal optical purity of **7** and **8** is 50% and the optimal *F* values are 2.0 and 0.5, respectively. Since hydrolysis is accompanied by an exchange of 0.5 atom % oxygen, the observed *F* values suggest an almost complete stereospecificity.

Acknowledgments. We are indebted to Professor D. R. Trentham for informing us of his independent work on chiral thiophosphates prior to publication, to Professors H. G. Floss and J. R. Knowles for useful discussions, and to J. F. Kozlowski for obtaining NMR spectra. This work was supported by NSF Grant PCM 79-11478 and NIH Grants RR 01077 and GM 26839.

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An Unexpected G–G Base Pairing Caused by the Coordination of Platinum(II) at the N(7) Position of 9-Ethylguanine

Sir:

The mechanism of the anticancer action of *cis*-$Pt(NH_3)_2Cl_2$[1] is thought to involve interaction of the drug with the bases of DNA.[2] There is good evidence for such an interaction both in vitro[3-6] and in vivo,[7-9] probably with guanine,[10] or at least with the guanine–cytosine base pair.[11] Suggested models include interstrand cross-linking,[12] an intrastrand clip,[13,14] an N(7)–O(6) chelate to guanine which interferes with hydrogen bonding,[15-17] and costacking of pairs of *cis*-Pt(II) complexes monofunctionally bound to adjacent bases on one strand.[18] The isolation of hydroxo-bridged Pt(II) amine complexes[19,20] and their peculiar interactions with DNA bases[21-23] lends credence to the postulate of an interaction of two platinum atoms at N(1) and O(6) of guanine[24] or alternately at N(7) and O(6).[25] All these models are based on the assumption that it is necessary to interfere with the replication process of DNA by interfering with the hydrogen-bonding sites used for producing base pairing between DNA strands. These models are consistent with recent ideas that cancer cells are deficient in their ability to excise defects from the DNA strand, and, thus, one can selectively kill a cancer cell chemically by introducing further defects into the DNA strand which cannot be excised.[2]

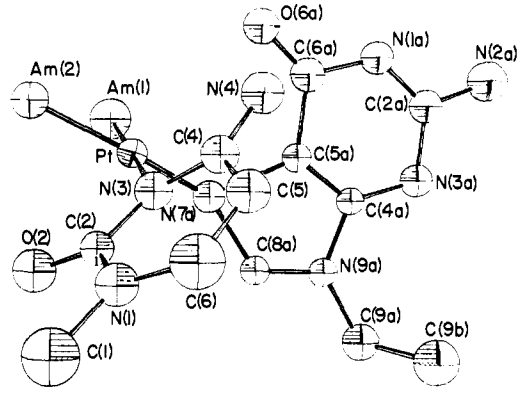

Figure 1. The molecular cations $[Pt(NH_3)_2(1\text{-MeC})(9\text{-EtG})]^{2+}$ or $[Pt(NH_3)_2(1\text{-MeC})(9\text{-EtG-H})]^{+}$.

The problem is that all crystallographic studies of model compounds of the *cis*-diammineplatinum(II) moiety combined with guanosine[26-28] show interaction at the N(7) site only, and this site

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