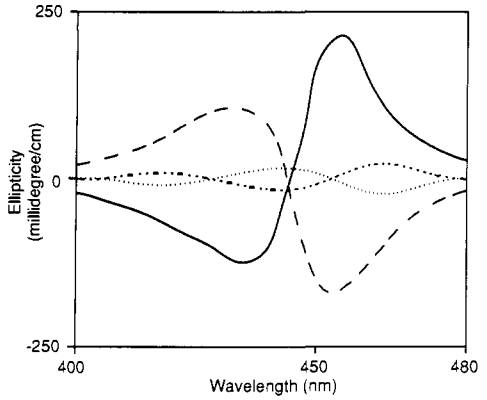

Figure 3. Induced circular dichroism spectra for 5 μM *trans*-H_2P_{agg} in the presence of 50 μM polypeptides at pH 4.5: (···) poly-D-glutamate, no NaCl added; (– – –) poly-D-glutamate, [NaCl] = 0.1 M; (–·–·–) poly-L-glutamate, no NaCl added; (—) poly-L-glutamate, [NaCl] = 0.1 M.

porphyrin concentrations studied, *trans*-AuP_{agg} appears to remain a monodispersed, intercalated porphyrin. A plot of the CD signal for the *trans*-AuP_{agg}/DNA complex vs concentration of the porphyrin is linear, as shown in Figure 2. Therefore, we conclude that, as observed in solution, *trans*-AuP_{agg} shows little or no tendency to aggregate, even on a DNA surface.

The aggregation model we have proposed for the production of large, conservative CD signals leads to the prediction that ds-DNA is not required by the process. In principle, any helical polymer of repeating, closely spaced negative charges to which *trans*-H_2P_{agg} or *trans*-CuP_{agg} binds should be capable of providing the template needed to produce such unusually large induced CD spectra. The prediction was tested, in part, by using a ss-DNA,[2] but a more convincing test is one in which no nucleic acid is used. To this end, we studied the binding of *trans*-H_2P_{agg} to poly-L- and poly-D-glutamate at pH 4.5, where the polypeptides have been reported as helical.[8] Although the complexes formed with these polypeptides are not as stable as the ones formed with nucleic acids (precipitates appear on standing), freshly prepared solutions produce large, conservative CD signals (Figure 3). It can be seen from Figure 3 that the phase of the signal reflects the helical sense of the polymer; the phases of induced CD of the porphyrin are reversed for the D versus L forms of polyglutamate. Above pH 6, polyglutamate becomes largely random coil albeit with some residual helical character.[9] The induced CD signals of *trans*-H_2P_{agg} with the random-coil polymer (pH 8) are correspondingly significantly smaller, and the phase disposition of the induced conservative porphyrin CD spectrum is opposite to that found when this porphyrin is bound to the same polymer but at conditions where it is predominantly α helix. This may indicate that the structure of the porphyrin aggregate on the random-coil polymer is different or that regions of the random-coil polymer have a helicity opposite to that of its "normal" low pH form.[9]

In conclusion, we offer these results as evidence for the spontaneous formation of supramolecular assemblies on helical templates by selected porphyrins. The tendency of a given porphyrin to aggregate in solution seems to be a useful indicator of the ability of that species to form highly extended assemblies. Kinetic studies to elucidate the mechanism of formation of these porphyrin supramolecular structures are underway.

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Chair-Form Six-Membered Ring Attached Diequatorially to Five-Coordinate Phosphorus. ^1H NMR and X-ray Crystallographic Study

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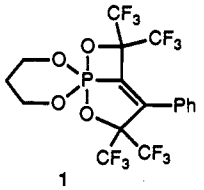
Studies reported over the past few years[1] have emphasized the preference of a 1,3,2-dioxaphosphorinane ring attached to five-coordinate phosphorus to be appended in an apical/equatorial rather than a diequatorial manner. Moreover, in nearly all instances the ring in question was found by ^1H NMR spectroscopy and X-ray crystallography to be in a boat or twist form rather than in a chair conformation. The exceptions were three nearly structurally identical phosphoranes with twist-chair conformations (X-ray results) arising as a result of intermolecular hydrogen bonding.[1a]

Up to now, no X-ray structures showing 1,3,2-dioxaphosphorinane rings attached diequatorially to five-coordinate phosphorus have been reported except for two molecules with the six-membered ring locked in the chair conformation.[2] The increase in energy required to coordinate the ring to phosphorus diequatorially is unknown, although activation free energies for pseudorotations via species with the 1,3,2-dioxaphosphorinane ring diequatorial have been determined.[3,1a,d] Furthermore, there is no information as to what conformation is of lowest energy, chair, twist, or boat.

We report here proof by ^1H NMR and X-ray crystallography for the *diequatorial* attachment of the 1,3,2-dioxaphosphorinane ring of **1** to five-coordinate phosphorus in an essentially trigonal-bipyramidal molecule. Moreover, the conformation of the ring is clearly a *chair* in the crystal; and this conformation is very largely, if not entirely, populated *in solution* as well.

Phosphorane **1** was prepared from reaction of 2-(2-phenylethynyl)-1,3,2-dioxaphosphorinane ($CH_2(CH_2O)_2PC{\equiv}CPh$) with $(CF_3)_2CO$ at −78 °C, a process well-known[4] for other three-coordinate alkynyl-substituted phosphorus compounds, and recrystallized from diethyl ether/pentane, mp 147–148 °C.[5]



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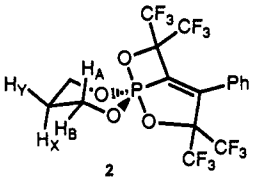

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(5) The ^{31}P NMR chemical shift for **1** in $CDCl_3$ at 1.4 ppm downfield from 85% H_3PO_4 is unusually low for a pentacoordinate phosphorane. However, a phosphorane containing the same bicyclic ring as **1**, but without the CF_3 substituents and with MeO in place of the 1,3,2-dioxaphosphorinane ring of **1**, showed a ^{31}P NMR shift at 11.7 ppm upfield from 85% H_3PO_4.[4a]

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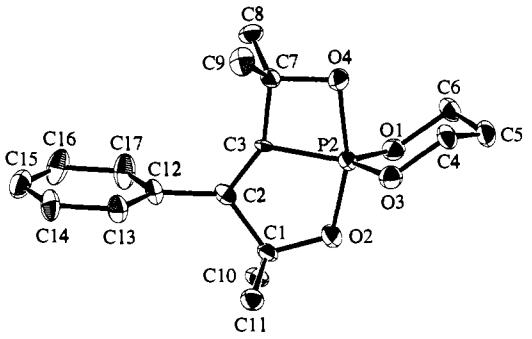


Figure 1. ORTEP drawing of X-ray structure **1**. Hydrogen and fluorine atoms are omitted for clarity.

Table I. Selected ^1H NMR Parameters for **1**[a]

Coupling Constants (Hz)							
J_{AP}	J_{BP}	J_{AX}	J_{AY}	J_{AB}	J_{BX}	J_{BY}	J_{XY}
5.6	25.0	11.5	2.9	−11.6	4.7	3.2	−14.8

Chemical Shifts, δ			
A	B	C	D
4.13	3.62	1.44	0.57

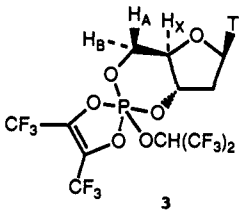
[a] At 300 MHz in C_6D_6. Simulated and iteratively refined by use of LAOCN3 program.

An ORTEP drawing of the X-ray structure of **1** is given in Figure 1.[6] The ligands about phosphorus are arranged in a distorted-trigonal-bipyramidal manner. Atoms O(1) and O(3) of the 1,3,2-dioxaphosphorinane ring *are attached equatorially* to phosphorus. Atoms O(1), O(3), C(3), and P(2) lie very close to the equatorial plane. Key bond angles in that plane include the following: O(1)–P(2)–O(3), 108.1 (3)°; O(1)–P(2)–C(3), 124.1 (3)°; C(3)–P(2)–O(3), 127.2 (3)°. The bond angles between O(2) and the atoms in the equatorial plane are within 5° of the 90° angles of an ideal trigonal bipyramid. The main deviation of the structure from trigonal-bipyramidal geometry arises from the incorporation of O(4) into a four-membered ring which moves the P(2)–O(4) bond away from perpendicularity to the equatorial plane (angle O(2)–P(2)–O(4), 162.3 (2)°).

Clearly, the six-membered ring in Figure 1 is in a *chair* conformation, contrary to Dreiding models and MNDO calculations,[7] which predict a half-chair conformation flattened about phosphorus. Unlike most 1,3,2-dioxaphosphorinane rings bonded in an apical/equatorial manner to five-coordinate phosphorus, the ring is not in a boat or twist conformation. The angle O(1)–P(2)–O(3) is reduced to 108.1°, which accommodates the puckering about P(2) required for a chair conformation. The P–O–C angles within the ring are also reduced to about 116° from the values around 120° found with twist- or boat-form apical/equatorial rings. Most notable among the other X-ray parameters is the exceptionally long P(2)–O(4) bond, 1.799 (5) Å, also found for a closely related phosphorane with a four-membered ring coordinated diequatorially to phosphorus.[4c] Apical P–O bonds average about 1.73 Å for 1,3,2-dioxaphospholane rings (five-membered rings) bonded in an apical/equatorial manner to five-coordinate phosphorus.[1d]

Table I displays the pertinent ^1H NMR data for **1** in C_6D_6. The well-separated 1,3,2-dioxaphosphorinane ring protons at 300 MHz, along with the phosphorus atom, constitute at an AA'MM'XYZ spin system, designated A, B, X, Y, and P in structure **2** and iteratively refined using the LAOCN3 computer program. The coupling constant pattern is totally parallel to that noted for many chair-form three- and four-coordinate 1,3,2-dioxaphosphorinanes.[8] Specifically, the couplings to phosphorus for the CH_2O protons, 25.0 and 5.6 Hz, reflect the contrasting H–C–O–P torsion angles of the equatorial (H_B) and axial (H_A) protons, respectively. Furthermore, J_{AX} is large (11.5 Hz), as expected for the antiperiplanar relationship of H_A and H_X. Consistent with structure **2**, J_{AY} (2.9 Hz), J_{BX} (4.7 Hz), and J_{BY} (3.2 Hz) are all relatively small. Moreover, the above pattern is in direct contrast to that found for five-coordinate phosphorus molecules with the 1,3,2-oxaza-[9] or -dioxaphosphorinane[1c,f,g] ring in a twist or boat conformation.

The above results have implications regarding the attachment of the five-coordinate phosphorus containing rings of **3**[1c] and **4**[7] studied previously. These phosphoranes, derived from thymidine,



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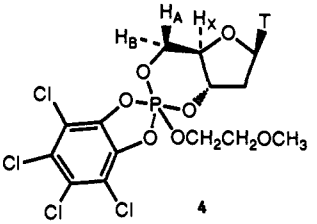

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are models for the transition state or intermediate in phosphodiesterase-catalyzed hydrolysis of cAMP. From the observed pattern of J_{HH} and J_{HP} values for protons A, B, and X, **3** was assigned a twist conformation in solution with the six-membered ring apical/equatorial on phosphorus.[1c] However, for **4** it was postulated[7] that a significant portion of molecules populate a ring which is attached diequatorially to phosphorus in a structure in pseudorotational equilibrium with the apical/equatorial form. Nonetheless, the coupling constants for H_A, H_B, and H_X are nearly identical for **3** and **4**.[1c,7] If indeed the diequatorial form of **3** and **4** is a chair conformation, as found for **1**, then the observed coupling constants for **3** and **4** *exclude the possibility that either can have the 1,3,2-dioxaphosphorinane diequatorial to any considerable extent.*

It should be pointed out that the conversion of the 1,3,2-dioxaphosphorinane ring of **2** to the boat or twist conformation does not move a p orbital lone pair on a ring oxygen into the equatorial plane. Therefore, the stabilizing equatorial oxygen lone pair pπ–dπ back-bonding with phosphorus,[10] available to the twist-form ring attached in an apical/equatorial manner, and first suggested by Trippett[3a] as the driving force for chair-to-twist conversion of apical/equatorial 1,3,2-dioxaphosphorinane rings of phosphoranes, *is not available to such rings attached diequatorially, regardless of conformation.*

Finally, it will be important to examine the structures of other phosphoranes in which syn-axial repulsions between the apical oxygen on phosphorus (O(4) in **2**) and the ring hydrogens axial on carbons of the 1,3,2-dioxaphosphorinane ring (on C(4) and C(6) of **2**) are not potentially reduced by the bending of bond P(2)–O(4) of **2** away from the six-membered ring. For such molecules the stability of the twist form may move closer to that of the chair.

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Note Added in Proof. After this communication was submitted, an X-ray structure was published of a five-coordinate dioxaphosphorinane with a *chair form*, six-membered ring attached in *apical–equatorial* manner to phosphorus (Hans, J.; Day, R. O.; Howe, L.; Holmes, R. R. *Inorg. Chem.* **1991**, *30*, 3132). This is the first example of such a ring in the chair conformation in

(6) Orthorhombic, space group *Pca*21; 3284 reflections obtained at −125 °C. Solved by direct methods, SDP/VAX package. $R_w = 0.078$.

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the *absence* of intermolecular hydrogen bonding. (See first paragraph of the present communication.)

Supplementary Material Available: Tables of crystal data, positional parameters, bond lengths and angles, and torsion angles for **1** (15 pages); listing of observed and calculated structure factors for **1** (8 pages). Ordering information is given on any current masthead page.

Laser Vaporization of Single-Stranded DNA. A Study of Photoinduced Phosphodiester Bond Scission

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One prerequisite for gas-phase analysis of large, fragile biomolecules is the molecular transfer of these species into the vapor state. This transfer has been accomplished for proteins,[1] duplex DNA,[2,3] and short oligonucleotides[4] via laser vaporization. Here a thin film of biomolecule and excess chromophore is irradiated with an intense laser pulse such that energy is deposited into the system so rapidly (10 ns) that molecular ejection occurs before thermal degradation begins. It is possible that laser vaporization may ultimately form the foundation for high-speed DNA sequencing wherein gel electrophoresis is replaced by mass spectrometry. In this scenario, a single-stranded dideoxy DNA sequencing product is molecularly vaporized by a pulsed laser, then ionized, and detected by mass spectrometry. We report in this communication the laser vaporization of a 17-base-long single-stranded oligonucleotide. We find that the extent of phosphodiester bond scission is dependent on the power of the laser pulse and, more importantly, that the extent of *molecular* vaporization scales with the laser pulse power. The significance of this result rests in the fact that vaporization of single-stranded DNA without strand scission is a crucial step for mass spectral based DNA sequencing.

To investigate the laser vaporization of single-stranded DNA, a mixture of a ^{32}P-labeled 17-mer and rhodamine 6G (1:17 000 molar ratio) is deposited onto a glass microscope slide. The resulting thin film will absorb green light by virtue of the rhodamine 6G dye chromophore.[5] The dried sample is then placed into a vacuum chamber (1×10^{-6} Torr) 10 mm from a piece of Whatman 3MM filter paper. A doubled Nd:YAG laser (532 nm, 7-ns pulse) is directed at the front of the sample, and the vaporized materials are collected on the filter paper. Figure 1 (panels A–C) shows an autoradiograph of the filter papers obtained upon exposing the 17-mer sample to laser pulses having powers of 130, 85, and 45 mJ/cm^2, respectively. Note that the ^{32}P-labeled material present on the filter paper is evenly distributed in a tightly focused spot, as is expected for molecular vaporization. Prior studies[2] have shown that the removal of macroscopic pieces of the mixture (spallation) results in a spotted or speckled appearance.

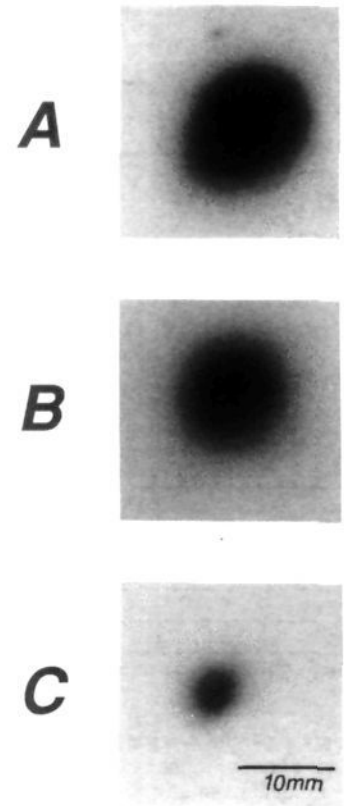

Figure 1. Autoradiograms of filters containing a vaporized oligonucleotide (17-mer, MW ≅ 5600) as a function of vaporization laser power: panel A, 130 mJ/cm^2; panel B, 85 mJ/cm^2; panel C, 45 mJ/cm^2.

While these results strongly suggest that spallation does not occur when rhodamine 6G is used, we cannot rule out the formation of small amounts of molecular dimer and trimer.

To further characterize the 17-mer vaporization products, the radioactive material on each filter is eluted by soaking in water and then analyzed by high-resolution polyacrylamide gel electrophoresis.[6] The sample that had been vaporized with a laser power of 45 mJ/cm^2 shows extensive strand scission, giving rise to strands of an average chain length of four nucleotides (Figure 2, lane 3). However, the samples vaporized at 130 and 85 mJ/cm^2 (Figure 2, lanes 1 and 2) display no observable strand scission, although some inorganic phosphate (P_i) is produced. The vaporized product should display less decomposition at high fluences because of collisional cooling. This results from the increased density of desorbed material resembling a free jet expansion.[3,7-9]

To gain insight into the chemical bond-breaking process observed in the 17-mer experiment, the vaporization of a simpler chemical species, [α-^{32}P]dATP, as a function of laser power was studied. Laser vaporization is carried out at laser fluences between 45 and 320 mJ/cm^2. The products of the vaporization process are collected on filter paper, analyzed by TLC, and compared to a series of standards,[10] and the amount of radioactivity in each of the components present is quantified (Figure 3). As predicted from the 17-mer experiments, the highest power levels result in little decomposition: at 320 mJ/cm^2 approximately 90% of the radioactivity present on the filter paper is recovered as intact dATP. As the laser power is reduced, several trends are observed:

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