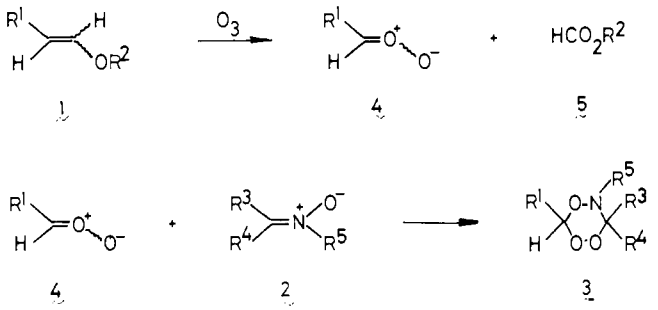
Scheme I



as mixtures of stereoisomers, e.g., compounds **3f-h,j,k**. In reality, the reaction of benzaldehyde *O*-oxide (**4b**) with nitrones **2a-c** afforded trioxazinanes **3f-h** as single isomers, whereas octanal *O*-oxide (**4c**) with nitrones **2a,c** gave the corresponding trioxazinanes **3j,k** as mixtures of isomers.[4] Since [3 + 3] cycloadditions between two 1,3-dipoles is predicted to be stepwise, unless one of the components is antarafacial, the trioxazinane isomer ratio is likely to be sensitive to the structures of either or both the carbonyl oxide and the nitrone. Although the cis and trans isomers of **3j** are formed in almost equal amounts, subsequent treatment of *cis*-**3j** [^{1}H NMR δ 5.65 (t, J = 5 Hz, H-3) and 6.46 (s, H-6)] with chlorosulfonic acid (0.1 equiv) in methylene chloride afforded *trans*-**3j** [^{1}H NMR δ 5.69 (s, H-6) and 5.82 (t, J = 5 Hz, H-3]. Under similar conditions, however, *trans*-**3k** [^{1}H NMR δ 4.1-4.2 (m, H-6) and 5.67 (t, J = 5 Hz, H-3)] was isomerized to *cis*-**3k** [^{1}H NMR δ 4.7-4.8 (m, H-6) and 5.45 (t, J = 5 Hz, H-3)].

In a nonparticipating solvent like methylene chloride, the carbonyl oxide **4**, generated in situ by selective ozonolysis of the vinyl ether **1**, reacted preferentially with the nitrone **2** to yield the corresponding 1,2,4,5-trioxazinane **3** (Scheme I). The alkyl formate **5**, coproduced from **1**, being a poor 1,3-dipolarophile,[5] did not combine with the carbonyl oxide. Ozonolyses of mixtures of the vinyl ether **1b** and nitrones containing 1 equiv of carbonyl compounds like benzaldehyde and benzophenone still gave the expected trioxazinanes **3** as the sole, isolable peroxidic products, albeit in reduced yield. Thus, for example, ozonolysis of β-methoxystyrene (**1b**) in the presence of a 1:1 mixture of nitrone **2d** and benzophenone afforded **3i** in 46% yield. In methanol, ozonolysis of a mixture of **1b** and nitrone **2d** gave the solvent derived α-methoxy hydroperoxide (55%) together with a small amount of **3i** (1%) consistent with more efficient capture of the intermediate carbonyl oxide by methanol.[5]

1,2,4,5-Trioxazinanes, as exemplified by derivative **3h**, have chemical properties similar to other stable six-membered cyclic peroxides, e.g., 1,2,4,5-tetroxanes. Thermolysis of **3h** for 8 h in refluxing benzene afforded a mixture of ring cleavage products, benzaldehyde (78%), octanal (78%), and benzaldoxime (53%), together with unreacted **3h** (11%). Treatment of **3h** with sodium ethoxide (13 equiv) in ethanol for 1 day at room temperature gave benzoic acid (93%), the nitrone **2c** (49%), and octanal (33%). Reduction of **3h** with triphenylphosphine proceeded very slowly at room temperature (only 20% **3h** reacted after 88 h) yielding almost quantitatively a clean product mixture of benzaldehyde and nitrone **2c**. Under similar conditions, **3h** did not react with thioanisole.

Preliminary attempts to extend the [3 + 3] cycloaddition strategy by utilizing other 1,3-dipoles have thus far been unsuccessful, neither 2,4,6-trimethylbenzonitrile oxide nor phenanthrium *N*-benzoylimide nor azoxybenzene captures carbonyl oxides as efficiently as nitrones under the reaction conditions described above.

Supplementary Material Available: Crystal data for **3e**, spectral data (^{1}H NMR) for **3a-m**, and tables of bond lengths, bond angles, fractional coordinates, and anisotropic vibration parameters (7 pages); table of observed and calculated structure factors (8 pages). Ordering information is given on any current masthead page.

(4) We have tentatively assigned the stereochemistry on the basis that in ^{1}H NMR spectra the equatorial proton would appear at a lower field compared with the axial one: Halls, P. J.; Jones, R. A. Y.; Katritzky, A. R.; Snarey, M.; Trepanier, D. L. *J. Chem. Soc. B* **1971**, 1320.

(5) (a) LaBarge, M. S.; Keul, H.; Kuczkowski, R. L.; Wallsch, M.; Cremer, D. *J. Am. Chem. Soc.* **1988**, *110*, 2081. (b) Nakamura, N.; Nojima, M.; Kusabayashi, S. *Ibid.* **1987**, *109*, 4969. (c) Mori, M.; Nojima, M.; Kusabayashi, S.; McCullough, K. J. *J. Chem. Soc., Chem. Commun.* **1988**, 1550.

A Remarkable Pericyclic Mechanism for Enzyme-Catalyzed P-C Bond Formation

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Recently,[1] we reported the isolation of the phosphorus-carbon bond-forming enzyme, PEP-phosphomutase, from *Tetrahymena pyriformis*. In *T. pyriformis* this enzyme plays a central role in 2-aminoethylphosphonate (AEP) biosynthesis through its catalysis of the first committed step involving C-P bond formation in the conversion of phosphoenolpyruvate (PEP) to phosphonopyruvate (PP). Since PEP and AEP are known to serve as precursors for a number of structurally diverse phosphonates,[2] the phosphoester-to-phosphonate rearrangement promoted by the phosphomutase might represent a common step in the biosynthesis of the phosphonate class of natural products.

Possible mechanisms for the PEP to PP rearrangement were suggested in our preliminary report.[1] These include a concerted sigmatropic phosphoryl migration, a stepwise double displacement route, and a stepwise cyclization-ring opening path through an oxaphosphatane intermediate (shown in Scheme I). We anticipated that an analysis of the stereochemical integrity of the migrating phosphoryl center would provide decisive information leading to elucidation of the mechanism for this important enzymatic transformation. Owing to a substantial driving force, the equilibrium between PEP and PP strongly favors PEP.[3] This feature coupled with the fact that procedures are known[4] for determining the stereochemistry of O-isotopically labeled thiophosphoenolpyruvate (TPEP) has led to a design of methodology to address the phosphomutase stereochemical problem which is based upon chiral [^{18}O,^{16}O]thiophosphonopyruvate (CTPP). Herein we report a solution to this problem involving the synthesis and configuration assignments of the separate enantiomers of CTPP, their phosphomutase-catalyzed isomerizations, and stereochemical analysis of the enantiomers of chiral [^{18}O,^{16}O$_2$]-thiophosphoenolpyruvate (CTPEP) which are products of these reactions.

The enantiomerically pure antipodes of CTPP were prepared by the sequence shown in Scheme II which advantageously utilizes HPLC separation of the diastereomeric phosphonamides **1**, derived

(1) Bowman, E.; McQueney, M.; Barry, R. J.; Dunaway-Mariano, D. *J. Am. Chem. Soc.* **1988**, *110*, 5575.

(2) Trebst, A.; Geike, F. Z. *Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1967**, *22*, 989. Horiguchi, M. *Biochim. Biophys. Acta* **1972**, *261*, 102. Barry, R. J.; Bowman, E.; McQueney, M.; Dunaway-Mariano, D. *Biochem. Biophys. Res. Commun.* **1988**, *153*, 177. Rogers, T. O.; Birnbaum, J. *J. Antimicrob. Agents Chemother.* **1974**, *5*, 121. Sato, H. In *Mycotoxins and Phycotoxins*; Steyn, P. S., Vleggaar, R., Eds.; Elsevier: Amsterdam, 1986; p 77.

(3) This is due to a much larger BDE for the P-O vs P-C bond. Unpublished studies conducted in collaboration with Profesor Jack Tossell suggest an ca. 20 kcal/mol energy difference between the trianionic forms of these substances.

(4) Sheu, K.-F.; Ho, H.-T.; Nolan, L. D.; Markovitz, P.; Richard, J. P.; Utter, M. F.; Frey, P. A. *Biochemistry* **1984**, *23*, 1779.

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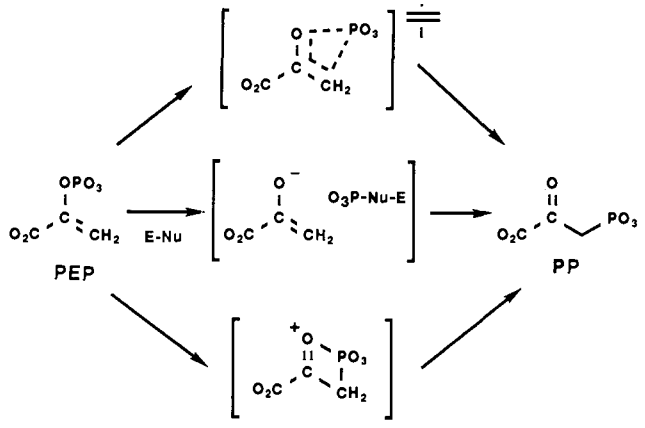
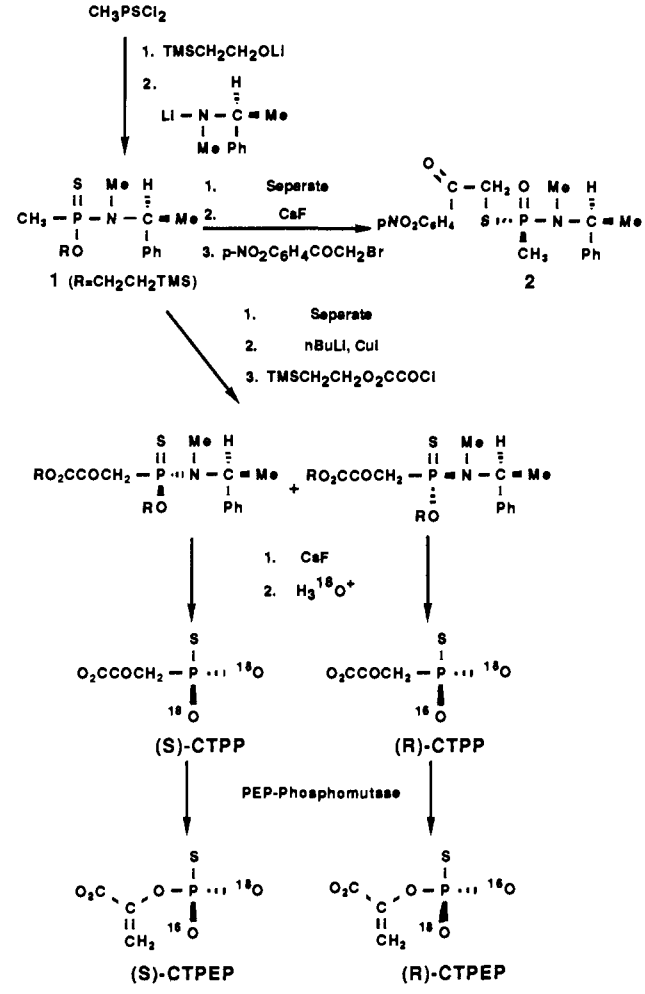
Scheme I



Scheme II



from $MePSCl_2$ by sequential TMS-ethanol and *N*-methyl-*N*-(*S*)-α-phenethylamine additions. Organocuprate coupling[5] to elaborate the pyruvoyl moiety is followed by fluoride induced bis-TMS-ethyl ester C–O bond cleavage and acid-catalyzed thiophosphonamide hydrolysis (inversion at phosphorus)[6] with $H_2{}^{18}O$. The absolute configurations at phosphorus in the CTPP enantiomers were determined by X-ray analysis[7] of the crystalline

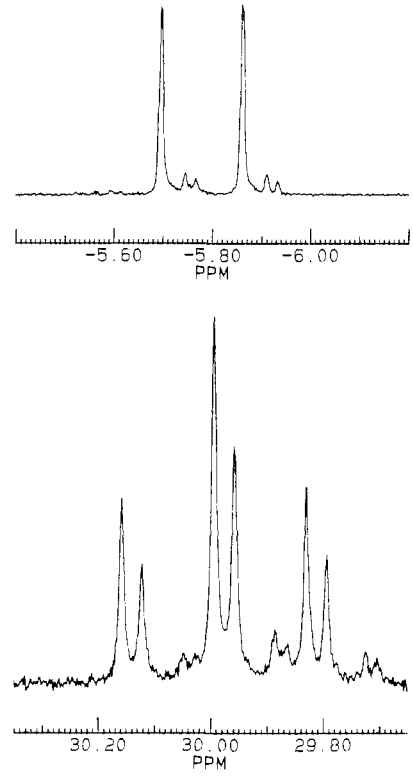


Figure 1. ^{31}P NMR spectrum of a 1:1 mixture of (S_P)-[$^{16}O_3$]ATPβS and (S_P)-[β-^{18}O]ATPβS derived from the CTPEP enantiomer arising from PEP-phosphomutase-catalyzed isomerization of (*S*)-CTPP (top γ-P region and bottom β-P region).

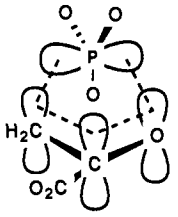
p-nitrophenacyl ester **2**, formed from the (S_P,S_C)-diastereomer of **1** (Scheme II).

The individual enantiomers of CTPP (0.18 mmol) were reacted (8 h, 25 °C) with PEP-phosphomutase (1.5 units) in 5.0 mL of 10 mM K_2PO_4 buffer (pH 8.0) containing 2.5 mM $MgCl_2$ and 0.8 mM dithiothreitol. Stereochemistry at phosphorus in the formed enantiomers of CTPEP was determined by stereospecific transfer of the thiophosphoryl moiety of these substances into the β-P position of ATP by known methods.[4] Configuration assignments to the (S_P)-[β-^{18}O]ATPβS diastereomers were made by use of ^{31}P NMR techniques.[8] The spectrum of (S_P)-[β-^{18}O]-ATPβS coming from the CTPEP enantiomer derived from (*S*)-CTPP (with equimolar (S_P)-ATPβS as reference) is shown in Figure 1. Clearly there is no ^{18}O-induced shift of the γ-P resonance in the spectrum of this substance, and the β-P resonance experiences an induced shift of the magnitude expected for the presence of ^{18}O at the nonbridging position. This analysis when coupled with the known stereochemical course of the CTPEP to (S_P)-[β-^{18}O]ATPβS conversion demonstrates that the (*S*)-enantiomer of CTPEP derives from (*S*)-CTPP and, in a similar fashion, that (*R*)-CTPEP comes from (*R*)-CTPP.

Thus, the reversible isomerization of phosphonopyruvate to phosphoenolpyruvate catalyzed by PEP-phosphomutase is stereospecific and occurs with inversion of configuration at phosphorus. This stereochemical result has important implications in terms of the mechanism for this phosphomutase-promoted isomerization. A double displacement pathway (Scheme I) for this process should display net retention at phosphorus since numerous observations have shown that enzymatic phosphoryl transfers to nucleophiles proceed with stereochemical inversion.[9] In addition, the two-step, oxaphosphatane mechanism should likewise occur with retention based upon precedent gained from studies of Wittig and related reactions which occur via analogous carba- and azaphosphatane intermediates.[10] In contrast, the

(5) Varlet, J.; Collignon, N.; Savinac, P. *Can. J. Chem.* **1979**, *57*, 3216.
(6) Cooper, D. B.; Harrison, J. M.; Inch, T. D. *Tetrahedron Lett.* **1974**, *31*, 2697. Harrison, J. M.; Inch, T. D.; Lewis, G. I. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1892.
(7) The crystallographic data were collected by Professor Herman Ammon and will be reported in a full paper on this subject.
(8) Webb, M. R.; Trentham, P. R. *J. Biol. Chem.* **1980**, *255*, 1775. Tsai, M.-D. *Biochemistry*, **1980**, *19*, 5310.
(9) Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877.

stereochemical course of the sigmatropic rearrangement involving concerted C to O 1,3-phosphoryl migration should be governed by orbital topology control.[11] As such, this four-electron, ground state pericyclic process would favor transition state **3** with a



3

Mobius topological orbital array corresponding to inversion at phosphorus.[12] Consequently, the observed stereochemical outcome strongly suggests that PEP-phosphomutase not only catalyzes a unique C-P bond-forming biosynthetic process but also employs a remarkably interesting pericyclic reaction mechanism.

Acknowledgment. These studies were aided by exceptionally competent input given by Professors Jack Tossell and Herman Ammon for which we are greatly indebted. Financial assistance was provided by NIH Grants GM-28688 (D.D.M.) and GM-27251 (P.S.M.) and a grant from the Center for Agricultural Biotechnology at The University of Maryland.

(10) Baraniak, J.; Kinas, R. W.; Lesiak, K.; Stec, W. J. *J. Chem. Soc., Chem. Commun.* **1979**, 940. Stec, W. J. *Acc. Chem. Res.* **1983**, *16*, 411. Gerlt, J. A.; Coderre, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 4531.

(11) Zimmerman, H. E. In *Pericyclic Reactions*; Marchand, A. P., Lehr, R. E., Eds.; Academic Press: 1977; Vol. 1, p 53.

(12) (a) For example, 1,3-sigmatropic carbon migration is known to occur with inversion of configuration at the migrating carbon (ref 12b): Berson, J. B. *Acc. Chem. Res.* **1968**, *1*, 152.

Synthesis and X-ray Structures of Compounds Having Very Short Phosphorus-Phosphorus Single Bonds: How Much of the Shortening in P-P Double Bonds Is Due to p-p π-Overlap?

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The classical model for double bonding in main group compounds involves a σ- and a π-bond.[1] Moreover, the difference in length between double and single bonds is often said to be due to the π-overlap. This simplistic picture has been modified to take account of the change in hybridization in the σ-bonding orbitals. For carbon-carbon bonds it is thought that about 70-75% of the shortening is due to p-p π-overlap, whereas 25-30% can be accounted for by the change in hybridization in the σ-orbitals from sp^3 to sp^2.[2] Consider now the case of the recently synthesized diphosphenes RP=PR[3,4] that have the P-P double bond distances of about 2.02 Å in comparison to P-P single bond lengths of ca. 2.22 Å. This contraction is also thought to be due mainly to a π-bond formed by side-on overlap of p-orbitals on the P atoms. However, the difference in hybridization between a diphosphane such as R_2PPR_2 and a diphosphene RPPR is even greater than that in carbon as the angles found in trivalent phosphorus compounds are considerably lower than tetrahedral values. The question that then arises is as follows: how much of the shortening in diphosphenes is due to the conventional p-p π-overlap and how much is due to the change in hybridization? In this paper an attempt is made to answer this question by the synthesis, spectroscopic, and structural characterization of the first examples of 1,1'-diboryldiphosphanes, the dimers $[PRBMes_2]_2$ (R = 1-Ad, **1**; R = Mes, **2**)[5] that may illustrate the relative contributions of rehybridization and p-p π-overlap to P-P double bond strength.

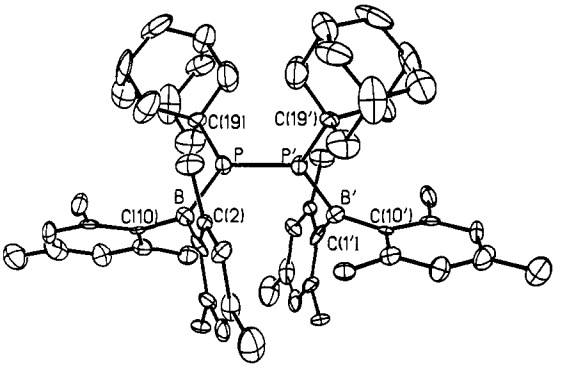

Figure 1. Computer-generated drawing of **1**, H atoms omitted for clarity. Selected bond distances (Å) and angles (deg) are as follows: P-P' = 2.109 (4), B-P = 1.852 (9), B-C(1) = 1.570 (12), B-C(10) = 1.599 (12), P-C(19) = 1.893 (8), C(1)-B-P = 119.3 (6), C(10)-B-P = 118.3 (6), C(1)-B-C(10) = 122.3 (7), B-P-C(19) = 120.5(4), B-P-P' = 118.3 (3), C(19)-P-P' = 120.6 (3).

The compounds **1** and **2** were synthesized,[6] in moderate yield, by the oxidation of the phosphinideneborate precursors $Li(Et_2O)_2PRBMes_2$ (R = 1-Ad or Mes) using $CrCl_3$. Both compounds were characterized by ^1H, ^{11}B, and ^{31}P NMR spectroscopy, and the X-ray crystal structure of **1**[7] is illustrated in Figure 1. Important structural parameters are given in the figure caption. There is a 2-fold rotation axis through the P-P' bond, which has a length of 2.109 (4) Å. (The incompletely refined structure of **2** also indicates a P-P distance of 2.11 Å.)[7] Both the boron and phosphorus centers are essentially planar (dihedral angle = 25.5°). There is also a large dihedral angle of 70.5° between the P and

(1) This view of double-bonding does not hold in the case of the heavier main group 4 analogues: Hitchcock, P. B.; Lappert, M. F.; Miles, S. J.; Thorne, A. J. *J. Chem. Soc., Chem. Commun.* **1984**, 480. Goldberg, D. E.; Hitchcock, P. B.; Lappert, M. F.; Thomas, K. M.; Fjelberg, T.; Haaland, A.; Schilling, B. E. R. *J. Chem. Soc., Dalton Trans.* **1986**, 2387. For calculations on disilenes, see: West, R. *Angew. Chem., Int. Ed. Engl.* **1987**, *12*, 1201. Raabe, G.; Michl, J. *Chem. Rev.* **1985**, *85*, 419 and references therein.

(2) March, J. *Advanced Organic Chemistry*, 3rd ed.; Wiley: New York, 1985; p 19. The bond lengths given in this reference are as follows: C-C single bonds; sp^3-sp^3 = 1.54 Å; sp^2-sp^2 = 1.48 Å, and C-C double bond in ethylene = 1.34 Å. Thus, the double bond is 13% shorter than a sp^3-sp^3 single bond, but the rehybridization is said to account for a 3.9% shortening (1.54-1.48 = 0.06 Å) or 30% of the overall contraction.

(3) Yoshifuji, M.; Shima, I.; Inamoto, N.; Hirotsu, K.; Higuchi, T. *J. Am. Chem. Soc.* **1981**, *103*, 4587.

(4) Cowley, A. H. *Polyhedron* **1984**, *3*, 389; *Acc. Chem. Res.* **1984**, *17*, 386. Cowley, A. H.; Norman, N. C. *Prog. Inorg. Chem.* **1986**, *34*, 1.

(5) 1-Ad = 1-adamantyl, Mes = 2,4,6-$Me_3C_6H_2$-.

(6) The synthesis of **1** is described here; compound **2** was synthesized similarly. Under anaerobic and anhydrous conditions $Mes_2BP(Ad)Li(Et_2O)_2$ (1.02 g, 1.79 mmol) in THF (35 mL) was added dropwise at 0 °C to a suspension of $CrCl_3$ (0.26 g, 1.80 mmol) in THF (30 mL). The reaction mixture was allowed to warm to room temperature and stirred overnight. All volatiles were removed under reduced pressure, and the brown-greenish residue was taken up in pentane (~150 mL). Filtration through Celite gave an intense yellow solution and a green-gray solid residue on the filter. Partial reduction of volume to ~85 mL, followed by slow cooling in a -20 °C freezer produced dark yellow crystals of **1**, suitable for X-ray structure determination. Further volume reduction, ~15 mL, gave a total yield of 0.41 g (48%) of **1**: mp 323-325 °C (dec); ^1H NMR (300 MHz, $CDCl_3$) δ = 6.73 and 6.64 (2 s, 2 H each = meta-H), 2.34, 1.98, 1.87 (br s, adamantyl) 2.23, 2.17, 1.55, 1.51 (s, ortho and para H); ^{11}B NMR (96.25 mHz, C_6D_6) δ = 49.3 (J_{B-P} = 50 Hz); ^{31}P NMR (145.73 MHz, hexane C_6H_{14}) δ = 2.8. **2**: 247-250 °C (dec); ^1H NMR (300 MHz, C_6D_6) δ = 6.6 (br s = meta-H), 3.2 q and 1.05 t (due to ethers of crystallization), 2.25, 2.07, 1.85 (br s ortho and para-H); ^{11}B NMR (96.25 MHz, C_6D_6) δ = 54.0; ^{31}P NMR (145.73 MHz, C_6D_6) δ = -22.3.

(7) Crystal data for **1**, at 130 K with Mo Kα (λ = 0.71069 Å) radiation; a = 20.953 (7) Å; b = 33.824 (6) Å; c = 13.596 (5) Å, orthorhombic, space group $Fdd2$; Z = 8 (dimers); 1113 unique observed ($I > 3\sigma(I)$) data R = 0.058. Crystal data for **2** could not be refined to an R value less than 0.19. This is primarily due to the inclusion of disordered Et_2O and, perhaps, pentane molecules in the crystal lattice. A reasonable model for the disorder was not found. The current state of refinement affords a P-P bond length of 2.11 Å with planar phosphorus centers which is in good agreement with the data for **1**. Crystal data for **2** at 130 K with Mo Kα (λ = 0.71069 Å) radiation: a = 19.801 (8) Å, b = 23.210 (8) Å, c = 24.198 (8) Å orthorhombic space group $Pbca$; Z = 8 (dimers); 2899 unique observed ($I > 2\sigma(I)$) data.

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