

remains unchanged in solution at room temperature. Fractional crystallizations resulted in different ratios of the three products. Each ratio remained unchanged after redissolving, confirming that the products are not interconverted and that the initial product mixture is formed under kinetic control. Similarly, compounds **9a**, **10a**, and **11a** remain unchanged in solution for several weeks at room temperature. Therefore, in contrast to analogous reactions of H-H and R₃Si-H bonds, reversible addition of tetrafluorocyclopropene is not observed.

As with our previous Pt chemistry,⁴ and in agreement with the suggestion of Binger (vide supra), no spectroscopic evidence for an intermediate η²-cyclopropene complex could be obtained in any of these reactions. Since the stereoselectivity of tetrafluorocyclopropene addition is identical with that found for kinetically controlled addition of the unambiguously σ-bonds of H-H and R₃Si-H to the same Ir(I) precursors,^{5,6} our results provide strongly suggestive evidence that metallacyclobutene formation occurs by analogous direct interaction of a cyclopropene C-C σ-bond with the metal and implicitly support such a pathway for the formation of their vinylcarbene valence tautomers.

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(8) **9b**: ¹⁹F NMR (C₆D₆) δ 77.2 (m, 2 F₁), 103.1 (m, F₂), 126.1 (m, F₃), J_{1,2} = 8, J_{1,3} = 19, J_{2,3} = 12, J_{1,P} = 2, J_{3,P} = 6 Hz; ³¹P{¹H} NMR (C₆D₆) δ -31.9 (m). **10b**: ¹⁹F NMR (C₆D₆) δ 97.5 (m, 2 F₁), 107.7 (m, F₂), 109.9 (m, F₃), J_{1,2} = 13, J_{1,3} = 18, J_{2,3} = 12, J_{1,P} = 5, J_{3,P} = 6 Hz; ³¹P{¹H} NMR (C₆D₆) δ -33.5 (m). **11b**: ¹⁹F NMR (C₆D₆) δ 78.3 (m, F₁), 98.1 (m, F_{1'}), 98.9 (m, F₂), 122.1 (m, F₃), J_{1,1'} = 205, J_{1,3} = 19, J_{1',3} = 17, J_{1,P} = 41, J_{1',P} = 40, J_{3,P} = 70 Hz (due to the low intensities of the peaks for this isomer, other ³¹P couplings could not be extracted from the spectrum); ³¹P{¹H} NMR (C₆D₆) δ -43.8 (m, P'), -47.3 (m, P).

Preparation of Phosphoenolpyruvate from D-(-)-3-Phosphoglyceric Acid for Use in Regeneration of ATP¹

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Adenosine 5'-triphosphate (ATP) is the most useful phosphoryl donor in enzyme-catalyzed synthesis.⁴ The most convenient procedure for in situ regeneration of ATP from ADP uses phosphoenolpyruvate (PEP) and pyruvate kinase (Scheme I). Commercial PEP is expensive and must usually be synthesized chemically for use in large-scale synthesis. Here we describe a practical procedure (the PGA method) that uses two inexpensive, commercially available enzymes to generate PEP in situ from the relatively inexpensive D-(-)-3-phosphoglyceric acid (3-PGA) (Scheme I).⁶

(1) Supported by NIH Grant GM 30367.

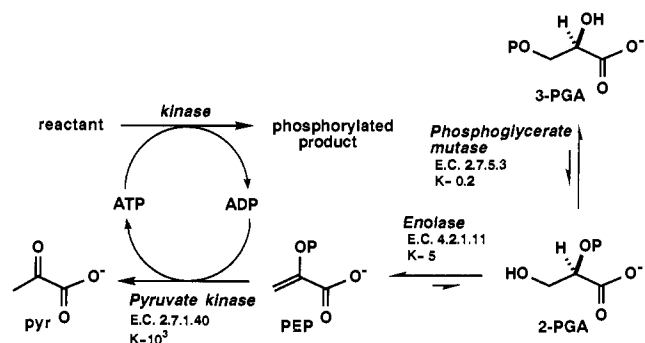
(2) Du Pont Fellow 1986-1987.

(3) NATO Postdoctoral Fellow 1988-1989 (administered by the Deutscher Akademischer Austauschdienst).

(4) Chenault, H. K.; Simon, E. S.; Whitesides, G. M. In *Biotechnology & Genetic Engineering Reviews*; Russell, G. E., Ed.; Intercept: Wimborne, Dorset, 1988; Vol. 6, Chapter 6.

(5) Hirschbein, B. L.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 3765.

Scheme I. Regeneration of ATP Using PEP^a



^a PEP can be synthesized in a separate chemical step or generated as shown from 3-PGA. P = phosphate; 3-PGA = D-(-)-3-phosphoglyceric acid; 2-PGA = D-(+)-2-phosphoglyceric acid; PEP = phosphoenolpyruvate; pyr = pyruvate.

Phosphoglycerate mutase⁷ converts 3-PGA to D-(+)-2-phosphoglyceric acid (2-PGA); enolase⁷ then forms PEP. Consumption of PEP by conversion of ADP to ATP, catalyzed by pyruvate kinase,⁷ drives the overall process.^{8,9}

We have tested the PGA method by using the reactions shown in Scheme II. CTP, GTP, and UTP are the nucleoside triphosphates needed for the syntheses of the most common nucleoside phosphate sugars used in Leloir pathway biosyntheses (reactions 1-4).¹⁰ Dihydroxyacetone phosphate (reaction 5) is used in reactions catalyzed by aldolases,¹¹ and arabinose 5-phosphate (reaction 6) is a precursor to 3-deoxy-D-manno-2-oculosonic acid 8-phosphate (KDO-8-P).¹² In all cases, procedures based on 3-PGA were as effective as and more convenient than procedures based on chemically synthesized PEP.⁵

The synthesis of CTP from CMP illustrates the PGA method.¹³ A suspension of 173 g of 3-PGA (barium salt, dihydrate, ~95%, 461 mmol) in 500 mL of water was stirred vigorously with ~600 mL of ion-exchange resin (Dowex 50W-X8, H⁺ form, 20-50 mesh) for 30 min at room temperature. The resin was removed by filtration and washed with four 100-mL portions of water.¹⁴ The combined, clear, pale-yellow filtrates were neutralized with solid KOH and used directly in the next step.

CMP (free acid, 71 g, 220 mmol), ATP·Na₂·3H₂O (1.33 g, 2.20 mmol), MgCl₂·6H₂O (51 g, 250 mmol), and triethanolamine (1.9 g, 10 mmol) were added to the solution of 3-PGA, and the pH was adjusted to pH 7.6 with 5 N KOH. The solution (total volume of 1 L) was degassed for 30 min with N₂; 2-mercaptoethanol (0.25 mL, 3 mmol) was added. The enzymes¹⁵ were then added, and

(6) From US Biochemical Corp.: PEP = \$4800/mol; 3-PGA = \$250/mol.

(7) Phosphoglycerate mutase: Ray, W. J., Jr.; Peck, E. J., Jr. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic: New York, 1972; Vol. VI, Chapter 12. Most commercial preparations of 3-PGA contain 2,3-diphosphoglyceric acid, an activator of phosphoglycerate mutase. Enolase: Wold, F. *Ibid.* Vol. V, Chapter 18. Pyruvate kinase: Kayne, F. J. *Ibid.* Vol. VIII A, Chapter 11.

(8) The isolation of PEP in 15-20% yield from 3-PGA using enzyme preparations from yeast has been reported: Ganti, T.; Csoka, A. *Magy. Kem. Foly.* **1975**, *81*, 335-6; *Chem. Abstr.* **1975**, *83*, 162155v. Csoka, A.; Ganti, T. *Hung. Teljes* **1974**, *8*, 931; *Chem. Abstr.* **1975**, *82*, 96491c.

(9) The three enzymes required are stable under the conditions used⁷ and are inexpensive (\$/1000 units): pyruvate kinase (0.3, Biozyme); enolase (4.75, Sigma); phosphoglycerate mutase (4.00, Sigma); 700 units of enzyme will convert 1 mol of reactants to products per day under assay conditions.

(10) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5635.

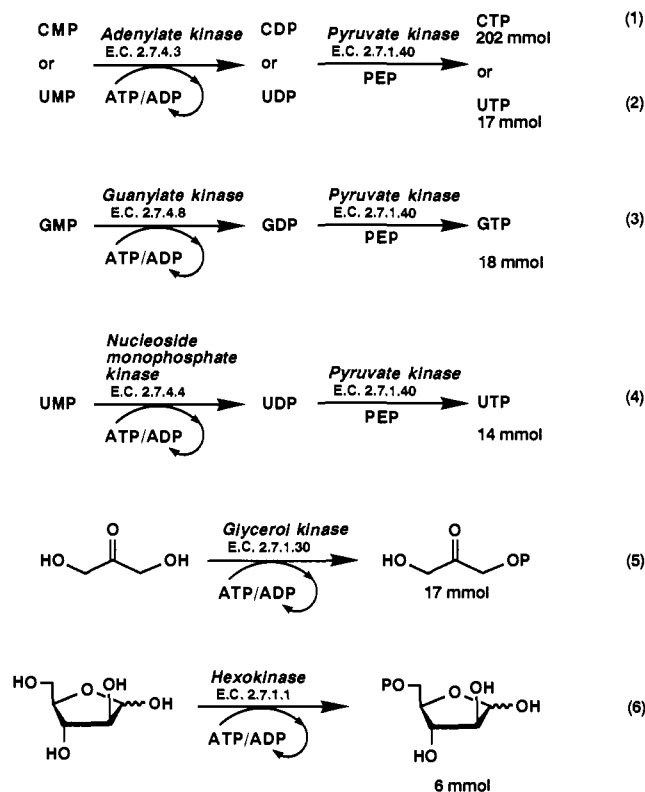
(11) Bednarski, M. D.; Simon, E. S.; Bischofberger, N.; Fessner, W.-D.; Kim, M.-J.; Lees, W.; Saito, T.; Waldmann, H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 627. Durrwachter, J. R.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175.

(12) Bednarski, M. D.; Crans, D. C.; Dicosimo, R.; Simon, E. S.; Stein, P. D.; Whitesides, G. M.; Schneider, M. *Tetrahedron Lett.* **1988**, *29*, 427.

(13) CMP is available from Miwon Foods Co., Ltd., Seoul, Korea (\$200/kg).

(14) The resin was regenerated with HCl by following the procedure recommended by the manufacturer.

Scheme II. Reactions Tested by Using the 3-PGA Method To Regenerate ATP According to Scheme I^a



^a Amount refers to quantity isolated. P = phosphate; CTP = cytidine 5'-triphosphate; UTP = uridine 5'-triphosphate; GTP = guanosine 5'-triphosphate.

the solution was stirred at 22–24 °C under a positive pressure of N₂. Addition of 3 M HCl using a pH stat controlled the pH at 7.5–7.8. After 48 h, consumption of HCl had ceased but ¹H NMR spectroscopy indicated that the reaction was not complete. An additional 32 g of 3-PGA (barium salt, 84 mmol) was converted to the K⁺ salt as described and added to the reaction mixture. After an additional 24 h, ¹H NMR spectroscopy indicated that the reaction was complete. A total of 130 mL of 3 N HCl had been consumed.

To isolate CTP, ~150-mL portions of the clear, pale-yellow reaction mixture were transferred into 12 500-mL polypropylene centrifuge tubes and 150 mL of absolute ethanol was added to each tube. A white precipitate formed immediately. The tubes were cooled in an ice bath for 15 min and then centrifuged (4 °C, 10000g, 10 min). The combined, sticky pellets were dissolved in 600 mL of water, the resulting solution was divided among six 250-mL centrifuge tubes, and 100 mL of absolute ethanol was added to each tube. The tubes were again cooled and centrifuged. Lyophilization of the combined pellets provided 145 g of an off-white powder containing 202 mmol of CTP according to enzymatic analysis¹⁶ (92% yield; 90% purity for CTP·K₃).¹⁷

(15) All enzymes were from Sigma: adenylate kinase (10000 units, lyophilized powder from chicken muscle), pyruvate kinase (5000 units, lyophilized powder from rabbit muscle), enolase (4000 units, lyophilized powder from yeast), and phosphoglycerate mutase (5000 units, suspension in ammonium sulfate from rabbit muscle). In this example, we used an excess of the enzymes because their cost is low compared with the value of the product.

(16) Keppler, D. In *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Bergmeyer, J., Grassl, M., Eds.; VCH: Weinheim, 1985; Vol. VII, p 432.

(17) The water content (7.14%) was determined by the Karl Fischer method. Thin-layer chromatography [poly(ethylenimine)-cellulose, eluant: 2.0 N HCOOH/2.0 N LiCl, 1:1, v/v] indicated that ATP was present in addition to CTP; neither CMP nor CDP was detected. Comparison of the ¹H and ³¹P NMR spectra with those of authentic CTP from Sigma indicated the presence of ~1% each of ATP, dipyruvate, 3-PGA, inorganic phosphate, and ethanol.

The primary advantage of the PGA method as a route to PEP is that it requires less time, produces less organic waste, and is more convenient than the chemical synthesis. Its starting materials are commercially available. It is, however, more expensive. The convenience outweighs the difference in cost for reactions carried out on a scale <1 mol. The convenience of the PGA method, along with its reliance on commercially available reagents, recommends it as a general technique for the in situ regeneration of ATP.

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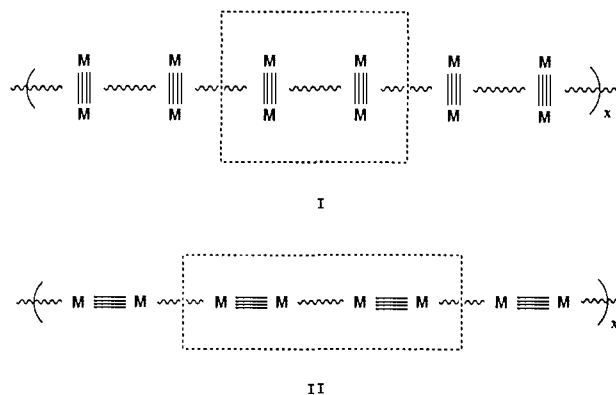
Electronic Coupling between Covalently Linked Metal–Metal Quadruple Bonds of Molybdenum and Tungsten

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Chemical and physical properties of one-dimensional materials that contain electronically coupled, redox-active transition-metal centers are of interest.¹ We report herein the synthesis and preliminary characterization of tetranuclear compounds of molybdenum and tungsten containing covalently linked M–M quadruple bonds. These compounds represent models for the dimeric subunits in hypothetical one-dimensional polymers of type I and II that may too show interesting electronic, optical, and magnetic properties.



The new tetranuclear compounds were synthesized according to the stoichiometric reaction shown in eq 1.² The tetranuclear $2\text{M}_2(\text{O}_2\text{C}-t\text{-Bu})_4 + \text{HO}_2\text{C}(\text{R})\text{CO}_2\text{H} \rightarrow [\text{M}_2(\text{O}_2\text{C}-t\text{-Bu})_3]_2(\mu\text{-O}_2\text{C}(\text{R})\text{CO}_2) + 2t\text{-BuCO}_2\text{H}$ (1)
M = Mo, W

(1) See, for example: (a) Marks, T. J. *Science* **1985**, *227*, 881. (b) Miller, J. S.; Epstein, A. J. *Prog. Inorg. Chem.* **1976**, *20*, 1. (c) Whangbo, M.-H.; Hoffmann, R. *J. Am. Chem. Soc.* **1978**, *100*, 6093.

(2) A typical procedure involves the reaction between $\text{M}_2(\text{O}_2\text{C}-t\text{-Bu})_4$ (ca. 500 mg), dissolved in dry toluene (10–15 mL) under an oxygen-free N₂ atmosphere, and the dicarboxylic acid (0.5 equiv). The solution is stirred at room temperature for ca. 1–3 days, during which time a finely divided microcrystalline precipitate forms. The precipitate is collected by filtration, washed with hexane (2 × 10 mL), and dried in vacuo to yield the crude compounds of type III or IV. Yields are ca. 90–95% for M = Mo and ca. 50–80% for M = W. Purification of the linked dinuclear compounds involves either recrystallization from pyridine or chromatography through Bio-Beads (Bio-Rad, S-X8) with THF eluent. The compounds reveal two types of O₂C-*t*-Bu ligands in the ratio of 1:2 in both ¹H and ¹³C NMR spectra assignable to pivalate ligands that are trans and cis to the bridging ligand, respectively. Satisfactory elemental analyses have been obtained for all compounds.