$C_{21}H_{71}N_7O_{62}P_2TiW_{17}$: C, 5.43; H, 1.54; N, 2.11. Found: C, 5.57; H, 1.49; N, 2.05.

Dawson Oxotitanium HPT 21 and Protonated Form 22. A solution of **16** (3.013 g, 0.662 mmol) in 25 mL of 0.25 M NaOAc buffer pH 5.25 at 60 °C was treated with potassium bis(oxalato)oxotitanate(IV) (333 mg, 0.940 mmol), and the resulting solution was stirred for 10 min. Then Me_3NHCl (4.655 g, 48.7 mmol) was added at 25 °C. The precipitate was washed with water and dried, giving 2.945 g (95%) of crude TMA salt. This was ion exchanged to K^+ salt **21**, the ^{31}P and ^{183}W NMR spectra of which were identical with those of the impurity observed in the preparation of **18a** and **18b** (see text). A sample of **21** was ion exchanged over Amberlyst 15 W resin (H^+ form) to give the protonated form **21**. The ^{31}P NMR spectrum of **21** thus obtained was identical with the ^{31}P NMR spectrum of the K^+ salt **19** obtained by Br_2 oxidation of **18d** (see previous experiment).

Acknowledgment. This research was supported by PHS Grant GM 27137 from the National Institute of General Medical Sciences. PHS Grant RR02336 and NSF Grant CHE-8411177 provided funds for the 300-MHz NMR spectrometer.

Functionalized Keggin- and Dawson-Type Cyclopentadienyltitanium Heteropolytungstate Anions: Small, Individually Distinguishable Labels for Conventional Transmission Electron Microscopy. 2. Reactions[1]

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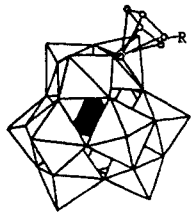
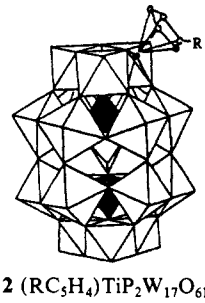
Received July 11, 1986

Abstract: Our goal is to develop a series of small, highly electron dense reagents that can be used to label substrate molecules covalently and chemoselectively for subsequent visualization by using conventional transmission electron microscopy (CTEM). Starting with the organic functionalized cyclopentadienyltitanium (CpTi) substituted Keggin- and Dawson-type heteropolytungstate (HPT) anions prepared in the accompanying paper, it was first established that the HPT unit as well as the Cp–Ti bond in those anions are stable under a variety of reaction conditions that lead to modification (esterification, acylation, reduction, reductive amination, oxidation, and cycloaddition reactions) of the organic appendage. A Diels–Alder reaction between either Keggin HPT diene **34** or Dawson HPT diene **18** and one of several substituted *N*-phenylmaleimides (**27–33**) was a versatile method for the attachment of a variety of protein-reactive groups to the HPT anions. Thus prepared were HPT maleimides **20** and **35**, bromoacetamides **21** and **36**, biotin derivative **22**, isothiocyanate **24**, and *N*-hydroxysuccinimide esters **37** and **40**. Additionally, the new heterobifunctional dienophiles **29**, **30**, **32**, and **33** should act as protein cross-linking agents, complementing those already available. Acylating agent **40** is noteworthy in that two Dawson HPT units are tethered in close proximity to each other in this reagent. By analogy to the EM image of "dimeric" HPT **23**, the EM image of **40** is expected to be recognizable morphologically as dumbells. HPT-labeled ATP derivative **42** was prepared by a reductive amination of Dawson benzaldehyde **10** with N^6-[[(aminohexyl)carbamoyl]methyl]ATP (Li salt). Both Keggin and Dawson HPTs are visible individually by using CTEM. Their stability in the electron beam is high.

The synthesis of a series of parent organic functionalized Keggin-type **1** and Dawson-type **2** cyclopentadienyltitanium (CpTi) heteropolytungstate (HPT) anions designed for use as



1 $(RC_5H_4)TiPW_{11}O_{39}^{4-}$



2 $(RC_5H_4)TiP_2W_{17}O_{61}^{7-}$

labels in conventional transmission electron microscopy (CTEM) is described in the accompanying paper.[2] Herein, we demonstrate that a variety of organic transformations may be effected on the organic portion of the HPT anions without affecting the HPT unit.[3] It is thus possible to introduce a single chemoselective protein-reactive group into the HPT anions that allows for the attachment of the EM label to biomolecules in a chemically well-defined manner. Among the reagents developed are several new heterobifunctional reagents that may also serve as protein crosslinking agents, complementing those already available.

Organic Functional Group Transformations on Derivatives of HPTs 1 and 2. The first objective was to determine the behavior of the HPT anions toward a variety of standard organic transformations. It was important to utilize where possible reaction conditions that gave a single product in near quantitative yield since no general method is available for separation of organic functionalized HPTs that differ only in the organic functional group. Throughout this work the product HPTs were normally converted into the slightly water soluble trimethylammonium

† University of Oregon.
‡ The Johns Hopkins University.

(1) A preliminary account has appeared: Keana, J. F. W.; Ogan, M. D.; Lü, Y.; Beer, M.; Varkey, J. *J. Am. Chem. Soc.* **1985**, *107*, 6714.
(2) Keana, J. F. W.; Ogan, M. D. *J. Am. Chem. Soc.*, preceding paper in this issue.
(3) Trimethyloxonium tetrafluoroborate effects methylation of the Keggin HPT $[(n\text{-}C_6H_{13})_4N]_3W_{12}PO_{40}$ on one of the bridging oxygen atoms: Knoth, W. H.; Harlow, R. L. *J. Am. Chem. Soc.* **1981**, *103*, 4265. A route to neutral, aryl-substituted heteropolyanions has recently been described: Siedle, A. R.; Lyon, P. A.; Hunt, S. L.; Skarjune, R. P. *J. Am. Chem. Soc.* **1986**, *108*, 6430.

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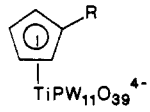
(TMA) salts for purification and analysis. These were then cation exchanged to the highly water soluble K^+ salts for spectral characterization and storage.

In the event, Keggin carboxylic acid **3** (H) gave ester **4** (H) upon Fischer esterification. The HPT moiety, itself a strong acid, catalyzed the reaction without effecting rupture of the Cp–Ti bond. HPT alcohol **5** (H) underwent acetylation to give acetate **6** (H).



3, R = $(CH_2)_5CO_2H$; (H) $\xrightarrow[\text{benzene, }\Delta]{\text{MeOH}}$ **4**, R = $(CH_2)_5CO_2Me$; (H), (TMA), and (K)

5, R = $(CH_2)_6OH$; (H) $\xrightarrow[\text{MeCN}]{Ac_2O}$ **6**, R = $(CH_2)_6OAc$; (H), (TMA), and (K)

7, R = $(CH_2)_6NH_3^+$; (H) $\xrightarrow[\text{MeCN}]{Ac_2O,\ py}$ **8**, R = $(CH_2)_6NHAc$; (TMA) and (K)

9, R = $(CH_2)_5CHO$; (K) $\xrightarrow[\text{NaOAc buffer pH 3.9}]{NaBH_3CN}$ **5** (K) + **5** (TMA)

9 (K) $\xrightarrow[\text{NH}_4\text{OAc buffer pH 6.5}]{NaBH_3CN}$ **7** (K) + **5** (K)

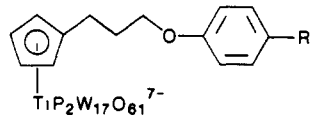
9 (TBA) $\xrightarrow[\text{NH}_4\text{Cl, MeCN}]{NaBH_3CN}$ **7** (TBA) ⟶ **7** (K)

9 (K) $\xrightarrow[H_2O]{CrO_3,\ H_2SO_4}$ **3** (K)

Abbreviations: (H), heteropolyacid; (K), K^+ salt; (TMA), Me_3NH^+ salt; (TBA), Bu_4N^+ salt.

Acetylation of HPT amine **7** (H) with Ac_2O failed in the absence of added base, likely because the amino group was protonated. The corresponding HPT amide pyridinium salt was formed in the presence of pyridine. However, this salt, like the HPT tetrabutylammonium (TBA) salts already described,[2] was difficult to convert to a water soluble salt and required ion exchange over acidic alumina.[2] Amide **8** (K) was eventually obtained in 36% yield.

Reduction of the Keggin aldehyde **9** (K) and Dawson aldehyde **10** (K) to the alcohols **5** (K) and **11** (K), respectively, was accomplished by using $NaBH_3CN$.[4,5] Alternatively, the HPT



11, R = CH_2OH; (K) and (TMA)

10, R = CHO; (K) $\xrightarrow{NaBH_3CN,\ pH\ 3.9}$ **11**

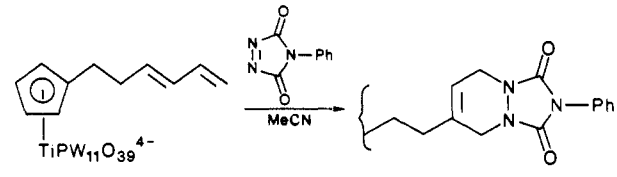
10, R = CHO; (K) $\xrightarrow[pH\ 6.5]{NaBH_3CN,\ NH_4OAc,}$ **12**, R = CH_2NH_2; (K) + **11** (K)

aldehydes underwent a reductive amination with $NaBH_3CN$ in the presence of NH_4OAc as the buffer. **9** (K) gave a 3:1 mixture of amine **7** (K) and alcohol **5** (K), both identical by NMR to samples prepared independently.[2] The ^{31}P NMR spectrum showed only one resonance. In general, ^{31}P NMR proved to be relatively insensitive to changes in functionality on the side chain of the HPTs. Reductive amination of Dawson aldehyde **10** (K) led to a mixture of amine **12** (K) and alcohol **11** (K) in a 9:1 ratio. A more efficient amination procedure involved the generation of Keggin aldehyde **9** (TBA) from the corresponding acetal[2] by passage of the acetal through acidic ion exchange resin. The resin effected deprotection without exchange of the TBA cations. After amination under anhydrous conditions,[6] the amine salt **7** (TBA) was metathetically exchanged to **7** (K) by using $Cs_2B_{10}Br_{10}$[7] followed by cation exchange. No ^1H NMR resonances due to alcohol **5** (K) were observed, essentially the only HPT present being amine **7** (K).

Keggin aldehyde **9** (K) underwent smooth oxidation in water to carboxylic acid **3** (K) with excess CrO_3–H_2SO_4. The ^{31}P NMR spectrum showed only one peak, demonstrating that the Cp–Ti bond was not attacked. This is remarkable since Br_2 in water is able to cleave this bond leading to the corresponding oxotitanium Keggin ion.[2] The fact that both the oxidizing agent and the Keggin ion are anionic in nature may explain the reluctance of the oxidizing agent to attack the Cp–Ti bond.

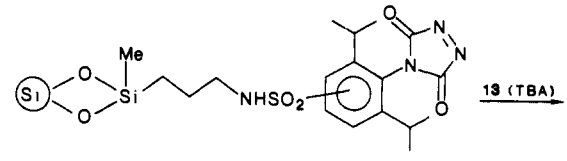
The reactions described above indicate that it should be possible to label substrates for EM visualization by one or the other of the following methods: acylation with HPT carboxylic acid **3**; amide formation with HPT amine **7**; esterification of HPT alcohol **5**, or a reductive amination reaction involving either HPT aldehydes **9** and **10** or HPT amine **7**. We next describe a versatile, complementary labeling approach in which a variety of molecules bearing a preformed protein reactive group may be attached quantitatively to an HPT ion in a manner which preserves the protein reactive group. A Diels–Alder reaction between a diene-containing HPT and a phenyl-substituted dienophile carrying a protein reactive group on the benzene ring constitutes the attachment method. Conventional organic synthesis may be used to prepare the protein reactive dienophile. Several suitably functionalized dienophiles (see below) are available commercially as protein crosslinking agents. The HPT EM label is then generated immediately prior to use without the need for involved HPT purifications or a separate activation step prior to labeling.

We began by allowing Keggin dienes **13** (TBA) and **13** (K) to react separately with the potent dienophile, 4-phenyl-1,2,4-triazoline-2,5-dione (Ph–TAD). Adducts **14** (TBA) and **14** (K) were formed, respectively, in quantitative yield.



13 (TBA) and (K) **14** (TBA) and (K)

A similar facile Diels–Alder reaction was observed between HPT diene **13** (TBA) and a suspension of the silica gel-immobilized 4-(2,6-diisopropyl-3(or 4)-sulfonatophenyl)-TAD **15**[8,9] in MeCN, leading to the novel HPT-labeled silica gel **16** in near



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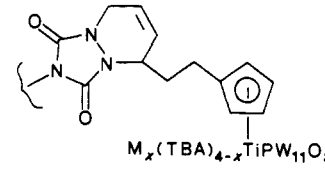


16

(4) We note that the introduction of ^3H into **10** should be possible by reduction with commercially available NaB^3H_3CN followed by reoxidation to the aldehyde. See: McMillen, D. A.; Volwerk, J. J.; Ohishi, J. I.; Erion, M.; Keana, J. F. W.; Jost, P. C.; Griffith, O. H. *Biochemistry* **1986**, *25*, 182.

(5) Lane, C. F. *Synthesis* **1975**, 135.

(6) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.

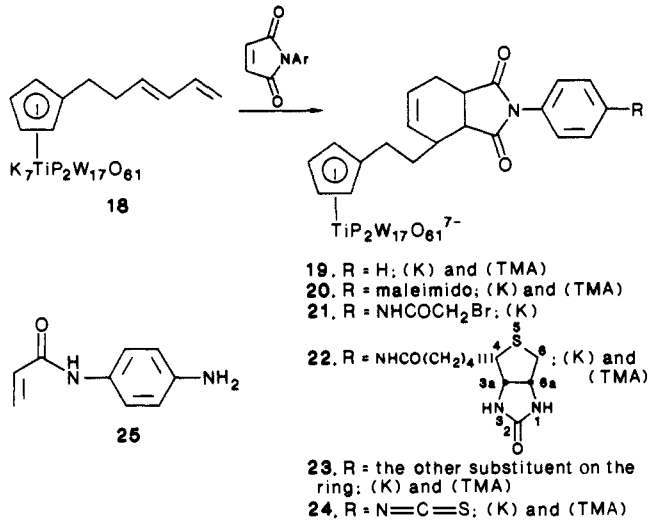
(7) Knoth, W. H.; Miller, H. C.; Sauer, J. C.; Balthis, J. H.; Chia, Y. T.; Muetterties, E. L. *Inorg. Chem.* **1964**, *3*, 159. We thank Dr. Knoth for a generous sample of this salt.

(8) Keana, J. F. W.; Guzikowski, A. P.; Ward, D. D.; Morat, C.; Van Nice, F. L. *J. Org. Chem.* **1983**, *48*, 2654.

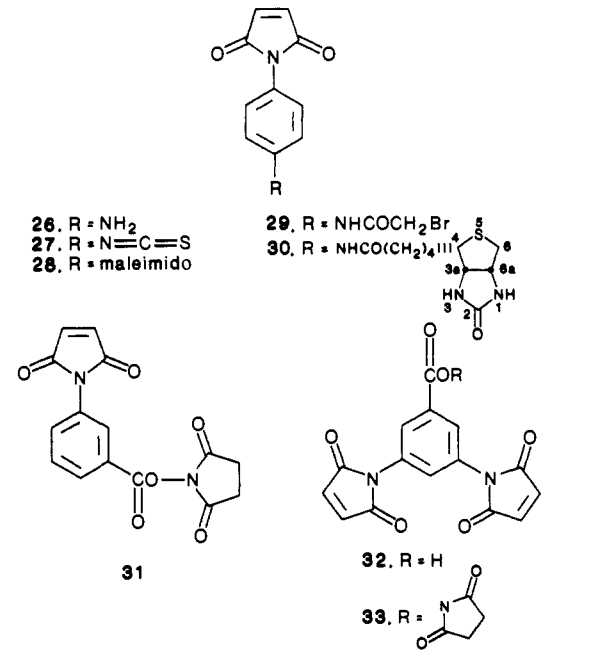
(9) We thank Dr. David D. Ward for performing this experiment.

quantitative yield.[10] No HPT could be detected in the filtrate by IR, ^1H, and ^{31}P NMR spectroscopy, although some Bu_4N^+ ion was present, presumably liberated by ion exchange of the HPT with Na^+ on the silica gel. Next, a 1.3:1 mixture of **13** (TBA) and the nondiene-containing HPT $(Bu_4N)_5Ti(=O)PW_{11}O_{39}$ (**17**)[2] was similarly treated with silica gel **15**. Oxo HPT **17** was recovered quantitatively from the filtrate while the diene-containing HPT **13** (TBA) was bound to the silica gel. This control experiment demonstrated that attachment to the silica gel was a consequence of the Diels–Alder reaction and not due to nonspecific interaction of the silica gel with the HPT moiety.

The use of less potent but more readily available dienophiles such as substituted *N*-phenylmaleimides would increase the versatility of the Diels–Alder route for the attachment of reactive groups to the HPTs. As a model, 1 equiv of *N*-phenylmaleimide[11] was allowed to react with Dawson HPT diene K^+ salt **18** in MeCN at 25 °C for 5 h, producing adduct **19** (K) quantitatively. The 360-MHz ^1H NMR spectrum was similar to those of other maleimide Diels–Alder adducts[8] and has been fully assigned with the aid of homonuclear decoupling experiments.[12] Acrylamide dienophile **25**, however, was not a satisfactory dienophile.



Introduction of Reactive Groups into the HPTs Suitable for Attachment to Biomolecules. Initial efforts[12] to prepare a series of para substituted phenyl TAD dienophiles bearing other reactive groups were not encouraging owing to the high reactivity of the TAD moiety toward nucleophiles, including traces of water.[8,13] In view of the successful synthesis of **19** by a Diels–Alder reaction, the preparation of several ring-functionalized phenylmaleimides was undertaken. Procedures for the preparation of maleimides **26**[14] and **27**[15] are given herein since the literature procedure for **26** did not work well in our hands,[16] and a procedure for **27** was not given. The maleimide-substituted alkylating agent, bromoamide **29**, was obtained by reaction of **26** with bromoacetyl bromide. The dienophiles **28**[17] and **31**[18] were available commercially. Acylation of **26** with *d*-biotin activated by reaction



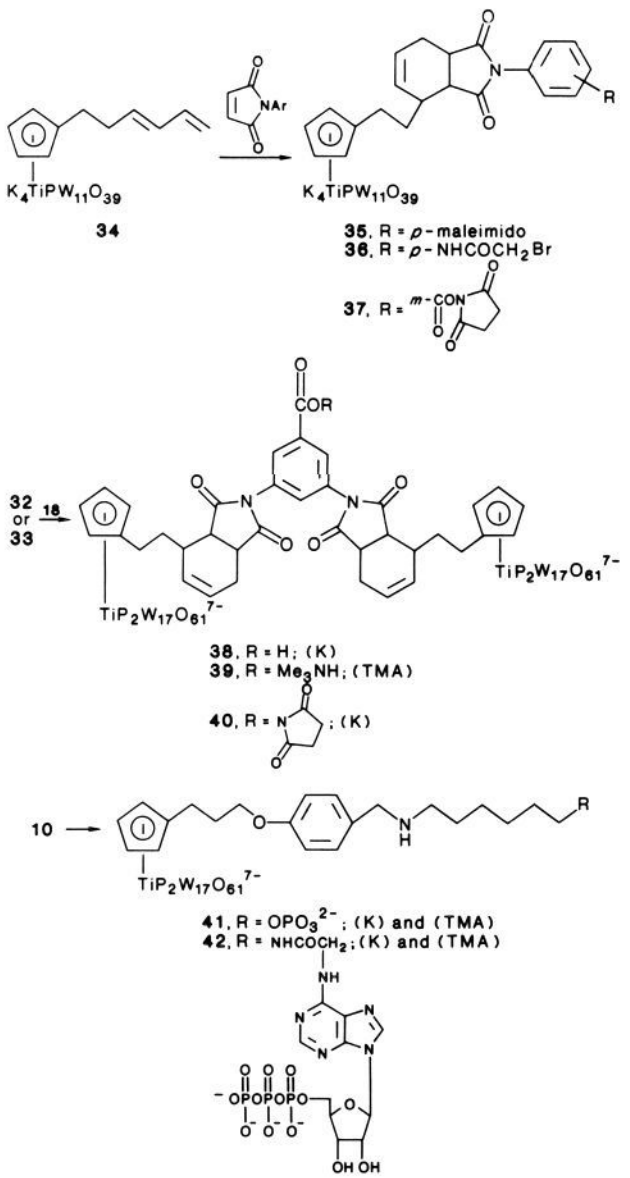
with methyl chloroformate[19] gave the biotinylated phenylmaleimide **30**. This target was chosen because biotin has enjoyed wide usage as a protein coupling agent for EM[20] owing to an extraordinarily low dissociation constant (10^{-15}) with avidin and streptavidin.[21] With an eye toward the development of a morphologically distinguishable EM label that contains two HPT groups in close proximity (see below), the bis maleimido carboxylic acid **32** and the corresponding activated ester **33** were prepared from 3,5-diaminobenzoic acid.

Dienophiles **27–33** were coupled to either Keggin HPT diene **34** or Dawson HPT diene **18**[2] or both by a Diels–Alder reaction analogous to that used to prepare adduct **19**. The reactions involving **28** and **30** were done, respectively, in anhydrous DMF and Me_2SO-d_6 owing to their low solubility in MeCN. Stoichiometric quantities of diene and dienophile were used in most instances. A fourfold excess of **28** was used in the synthesis of monoadducts **20** and **35** in order to minimize formation of the corresponding bis adducts (e.g., **23**). Thus, adducts **35–37** were obtained from Keggin HPT diene **34** while adducts **19–24** and **38–40** were obtained from Dawson HPT diene **18**. Partial hydrolysis of the active ester grouping in adducts **37** and **40** was observed, likely owing to the tendency of the HPTs to retain water. In the case of isothiocyanate adduct **24**, it was necessary to pretreat the Dawson diene **18** MeCN solution with 2 equiv of phenylisothiocyanate (scavenges water) prior to addition of maleimide **27** so that the isothiocyanate residue in **24** was preserved.

Biologically interesting ligands may be labeled with an electron dense HPT through use of the reductive amination procedure already described. Our objective was a labeled ATP derivative that could be used for the EM localization of ATP binding sites in certain proteins. The Dawson-labeled benzaldehyde **10** (K)[2] was chosen as the carbonyl component owing to the known tendency of benzaldehydes to form relatively stable Schiff bases with primary amines.

Suitable conditions were first worked out in a model reaction between aldehyde **10** (K), 6-aminohexyl phosphate, and $NaBH_3CN$ in aqueous buffer, giving amine **41** which was analyzed

(10) EM localization of the HPT residues on the silica gel is under investigation.
(11) Cava, M. P.; Deana, A. A.; Muth, K.; Mitchell, M. J. *Organic Synthesis*; Wiley: New York, 1973; Collect. Vol. 5, p 944.
(12) Ogan, M. D., Ph.D. Dissertation, University of Oregon, 1984.
(13) Capuano, L.; Muller, K. *Chem. Ber.* **1977**, *110*, 1691. Dao, L. H.; Mackay, D. *Can. J. Chem.* **1979**, *57*, 2727.
(14) Sippel, T. O. *Histochem. J.* **1973**, *5*, 413.
(15) Augustin, M.; Werndl, R.; Koehler, M.; Ruettinger, H. H. *Pharmazie* **1978**, *33*, 191. Maksudov, N. K. *Uzb. Khim. Zh.* **1976**, 39; *Chem. Abstr.* **1977**, *86*, 16610k.
(16) Another potential route to **26**, namely, selective reduction of the nitro group of the readily available *p*-nitrophenylmaleimide failed under a variety of conditions. Invariably, preferential reduction of the maleimide double bond was observed.
(17) Moore, J. E.; Ward, W. H. *J. Am. Chem. Soc.* **1956**, *78*, 2414. Chang, F. N.; Flaks, J. G. *J. Mol. Biol.* **1972**, *68*, 177.
(18) Kitagawa, T.; Aikawa, T. *J. Biochem.* **1976**, *79*, 233 and 236.
(19) Green, N. M.; Konieczny, L.; Toms, E. J.; Valentine, R. C. *Biochem. J.* **1971**, *125*, 781.
(20) See, for example: Heitzmann, H.; Richards, F. M. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3537. Wallace, B. A.; Richards, F. F.; Engelman, D. M. *J. Mol. Biol.* **1976**, *107*, 255. Bayer, E. A.; Skutelsky, E.; Wilchek, M. *Methods Enzymol.* **1979**, *62*, 308.
(21) Hsu, S. M.; Raine, L.; Fanger, H. *J. Histochem. Cytochem.* **1981**, *29*, 577.



as the TMA salt. Then **10** (K) was similarly treated with N^6-[[(aminohexyl)carbamoyl]methyl]adenosine 5′-triphosphate (Li salt) and $NaBH_3CN$ to give HPT-derivatized ATP **42**, accompanied by a small amount of benzyl alcohol **11**. Unlike our experience with mixtures of other HPTs (**1** and **2**) that differed only in the R group, chromatographic separation of **11** and **42** was possible. The ^{31}P NMR spectrum of **42** (K) indicated that about 30% had undergone hydrolysis to the corresponding HPT adenosine diphosphate (ADP) derivative.

pH Stability of Keggin and Dawson HPTs. By analogy to Knoth's[22] results, the new Keggin HPTs **1** herein described are not expected to be stable in aqueous solution above pH 7, a point at which the HPT unit itself begins to decompose. Pope[23] reported that while *n*-$BuSnPW_{11}O_{39}^{4-}$ is stable from pH 4 to 6, the analogous compound in the Dawson series, *n*-$BuSnP_2W_{17}O_{61}^{7-}$, is stable from pH 2 to 8. His work suggested that the new Dawson HPT labels **2** were likely to show improved stability over the Keggin labels **1** at higher pH values.

The pH stability of the parent Dawson HPT $K_7CpTiP_2W_{17}O_{61}$ (**2**, R = H)[2] was determined by observing the ^{31}P NMR spectra of buffered solutions (H_2O, D_2O, 1:1) at several pH values over time. The buffers were purposely of insufficient molarity to compensate fully for base consumption during the decomposition of **2** (R = H), thus the pH of the solution decreased until decomposition ceased. After 1 week, the spectra revealed that some **2** (R = H) was still present in the solutions which initially were pH 8.85 and 9.75. The final values for these solutions were pH 8.41 and 8.61, respectively. A sample initially at pH 7.95 showed no evidence of decomposition over a 1-week period. The upper limit of pH stability of **2** (R = H), corrected for the presence of deuterium in the solvent, is therefore about pH 8.2.

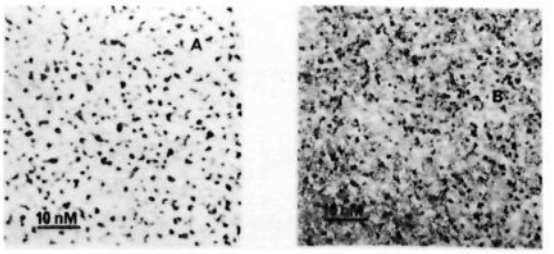


Figure 1. Electron micrograph of (A) Dawson benzaldehyde **10** (K) and (B) parent Dawson HPT **2** (R = H) on thin carbon films using a Philips 420 EM with a ST lens at 40 kV. Magnification 10^6.

Behavior of the HPT Labels in the Electron Microscope. That the Dawson HPTs are visible individually by using conventional transmission EM is demonstrated by the micrographs of aldehyde **10** (K) and the parent Dawson **2** (R = H) shown in Figure 1. The dense dots which are the images of the individual HPT anions are clearly detectable above the background. Similar micrographs were obtained in the Keggin series. Figure 2 shows a focal series with a change of $\Delta f = 75$ nm between successive micrographs. From these micrographs the size of Dawson HPT **2** (R = H) is estimated to be 1.0–1.5 nm. This is consistent with a size estimate that can be made from available X-ray crystallographic data,[24] namely, an ellipsoid of about 1.0 × 1.5 nm.

The position stability and apparently the chemical stability of HPT **2** (R = H) in the electron beam is excellent (Figure 3). The specimen was subjected to a beam current that gave satisfactory density in 2 s. The two exposures, taken 5 min apart, are indistinguishable.

A micrograph showing the morphologically unique dumbell image of "dimeric" HPT **23** (K) was included in our communication.[1] Functionalized analogues **38**–**40** are expected to appear similarly in electron micrographs. It should thus be possible to distinguish the "monomeric" HPT labels from the "dimeric" labels when each is used to label different components of a complex system such as a ribosome. These exciting possibilities are currently under investigation.

Experimental Section[25]

(Eicosa-μ-oxoundecaoxoundecatungstate)tetra-μ-oxo[(1,2,3,4,5-η)-1-[6-methoxy-6-oxohexyl]-2,4-cyclopentadien-1-yl][μ_{12}-[phosphato(3−)-*O*:*O*:*O*′:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴:*O*‴]]titanate(4−), Tetrakis(*N*,*N*-dimethylmethanamine) (4, TMA), 4 (H), and 4 (K). A solution of carboxylic acid **3** (H) (103 mg, 35 μmol) in 2 mL of MeOH and 0.5 mL of benzene was refluxed while 0.5 mL of solvent was removed by azeotropic distillation. After 2 h the solvent was removed giving ester **4** (H) quantitatively, as shown by ^1H NMR. This was dissolved in 5 mL of water, and a solution of Me_3NHCl (103 mg, 1.08 mmol) in 2 mL of water was added. The precipitate was crystallized from water, giving 83 mg (74%) of **4** (TMA) as orange crystals. Anal. Calcd for $C_{24}H_{57}N_4O_{41}PTiW_{11}$: C, 9.12; H, 1.82; N, 1.77. Found: C, 9.09; H, 1.89; N, 1.79.

A 62-mg sample was cation exchanged to give 62 mg of **4** (K): ^1H NMR δ 1.16–1.88 (m, 6), 2.34 (t, 2), 2.95 (t, 2), 3.56 (s, 3), 6.49 and 6.66 (AA′BB′, 4); ^{31}P NMR δ −14.01 (s).

HPT Acetate Esters 6 (H), 6 (TMA), and 6 (K). A suspension of HPT alcohol **5** (H) (67.5 mg, 23 μmol) in 2 mL of MeCN containing

(22) Knoth, W. H. *J. Am. Chem. Soc.* **1979**, *101*, 759.
(23) Pope, M. T.; Zonnevijlle, F. *J. Am. Chem. Soc.* **1979**, *101*, 2731.
(24) Dawson, B. *Acta Crystallogr.* **1953**, *6*, 113. D'Amour, V. H. *Acta Crystallogr., Sect. B Struct. Crystallogr. Cryst. Chem.* **1976**, *B32*, 729.
(25) The preamble to the Experimental Section of ref 2 applies here except that NMR spectra were measured in D_2O unless otherwise stated. Preparations of the starting HPTs used herein are given in the accompanying paper.[2]

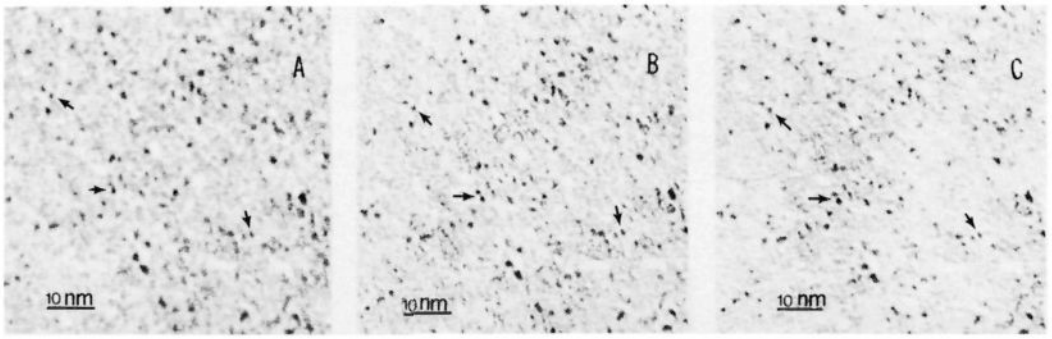


Figure 2. A focal series with a change of Δf = 75 nm between successive micrographs of **10** (K). The dimension of **10** (K) is about 1.0–1.5 nm. Magnification 10^6.

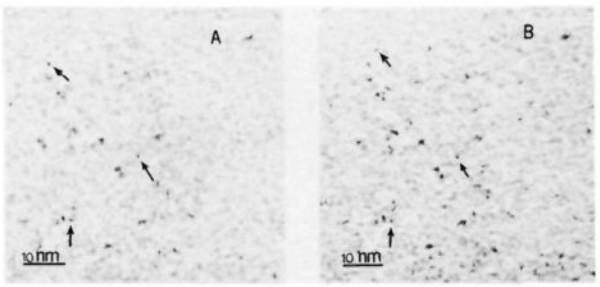


Figure 3. Electron micrograph of **2** (R = H) deposited on a thin carbon film. Exposure (b) was taken 5 min after exposure (a) with continuous irradiation at a beam current that gave satisfactory density in 2 s. Magnification 10^6.

Ac_2O (32.5 mg, 32 μmol) was stirred at 25 °C for 90 min and then evaporated to dryness. The ^1H NMR showed a quantitative formation of **6** (H). This was dissolved in 2 mL of water, and Me_3NHCl (467 mg, 4.89 mmol) was added. The precipitate was crystallized from water, giving ester **6** (TMA) as orange crystals. Anal. Calcd for $C_{25}H_{59}N_4O_{41}PTiW_{11}$: C, 9.46; H, 1.87; N, 1.77. Found: C, 9.51; H, 1.98; N, 1.87.

A portion was cation exchanged to **6** (K): ^1H NMR δ 1.20–1.95 (m, 8), 2.07 (s, 3), 2.99 (t, 2), 4.05 (t, 2), 6.54 and 6.70 (AA′BB′, 4); ^{31}P NMR δ −14.03 (s).

HPT Acetamides 8 (TMA) and 8 (K). To a solution of amine **7** (H) (189 mg, 62 μmol) in 2 mL of MeCN was added 86 mg (1.09 mmol) of pyridine and 75 mg (0.73 mmol) of Ac_2O, and the resulting suspension was stirred for 90 min at 25 °C. The solvent was removed, and the residue was dissolved in 2.5 mL of water–MeCN 3:1 and placed on a column (5 mm × 3 cm) of acidic alumina packed in the same solvent. The column was washed with water, and then the HPT was eluted with 1 M NaOAc buffer pH 6.2. The eluent was treated with Me_3NHCl (240 mg, 2.51 mmol); and the precipitated **8** (TMA) was washed with water, dissolved in hot water, and ion exchanged to give 69 mg (36%) of **8** (K): ^1H NMR δ 1.30–1.60 (m, 8), 1.92 (s, KOAc imp), 1.94 (s, 3), 2.98 (t, 2), 3.08 (t, 2), 6.53 and 6.70 (AA′BB′, 4); ^{31}P NMR δ −13.12 (s, oxo imp), −13.97 (s). The Ti=O oxo impurity resulted from partial base decomposition due to the presence of some KOAc. The analytical specimen of **8** (TMA) was prepared by reprecipitation of this salt. Anal. Calcd for $C_{25}H_{60}N_5O_{40}PTiW_{11}$: C, 9.47; H, 1.91; N, 2.21. Found: C, 9.28; H, 1.80; N, 2.16.

5 (TMA), 5 (K), and 6 (H) from the Reduction of Keggin Aldehyde 9 (K) with $NaBH_3CN$. A solution of **9** (K) (60 mg, 20 μmol) and 26 mg (0.41 mmol) of $NaBH_3CN$ in 1.0 mL of 0.12 M NaOAc buffer pH 3.9 at 25 °C was allowed to stir for 20 h, and then Me_3NHCl (31 mg, 0.33 mmol) was added. The precipitated **5** (TMA) was washed with water and then ion exchanged to give 55 mg (91%) of **5** (K). A 44-mg portion was ion exchanged to the HPT acid form **5** (H) and then acetylated as described above to give **6** (H). **5** (K) and **6** (H) were identical (^1H and ^{31}P NMR) with independently prepared samples.

11 (K) and 11 (TMA) from the Reduction of Dawson Aldehyde 10 (K) with $NaBH_3CN$. **10** (K) (71 mg, 15 μmol) was reduced as described above with 12 mg (0.19 mmol) of $NaBH_3CN$ to give 58 mg (83%) of **11** (K) as an orange powder: ^1H NMR δ 2.14 (pent, 2), 3.13 (t, 2), 4.12 (t, 2), 4.53 (s, 2), 6.47 and 6.64 (AA′BB′, 4); ^{31}P NMR δ −10.12 (s), −13.35 (s). An analytical specimen of **11** (TMA) was prepared by crystallization from water. Anal. Calcd for $C_{36}H_{87}N_7O_{63}P_2TiW_{17}$: C, 8.89; H, 1.80; N, 2.02. Found: C, 8.78; H, 1.71; N, 2.02.

7 (K) from Reductive Amination of 9 (K). A solution of **9** (K) (76 mg, 25 μmol) and $NaBH_3CN$ (22 mg, 0.35 mmol) in 1 mL of 0.25 M NH_4OAc buffer pH 6.5 was stirred at 25 °C for 119 h, and then Me_3NHCl (73 mg, 0.6 mmol) was added. The precipitate was ion exchanged to the orange K^+ salt (75 mg, 95%). The ^1H NMR spectrum revealed this to be a 3:1 mixture of amine **7** (K) and alcohol **5** (K).

12 (K) from Reductive Amination of 10 (K). A solution of **10** (K) (18 mg, 3.8 μmol) in 0.1 mL (200 μmol) of 2 M NH_4OAc buffer pH 6.5 at 25 °C was treated for each of 5 days with 0.06 mg of $NaBH_3CN$ dissolved in 20 μL of buffer (total added was 0.3 mg, 4.8 μmol). Then Me_3NHCl (5 mg, 52 μmol) was added. The precipitate was collected, washed twice with water (0.5 mL), and then ion exchanged to **12** (K) (17 mg, 92%). The ^1H NMR spectrum indicated that the product was a 9:1 mixture of amine **12** (K) and alcohol **11** (K).

7 (TBA) and 7 (K) from Reductive Amination of 9 (TBA). A 310-mg sample of the dimethyl acetal[2] of **9** (TBA) in MeCN was passed over 30 g of Dowex 50W 4X (H^+ form), giving 300 mg of aldehyde **9** (TBA) as a yellow solid which was dried (60 °C, 0.005 mm) overnight. A 130-mg sample (34 μmol) was dissolved in 2 mL of MeCN, placed over 3-Å molecular sieves (263 mg), and treated with NH_4Cl (12 mg, 0.23 mmol) and $NaBH_3CN$ (4.8 mg, 76 μmol). The suspension was stirred for 136 h and then filtered. Evaporation of the filtrate gave 163 mg of a yellow solid which was carefully triturated with MeCN, leaving a white solid. The yellow solution was evaporated to give 123 mg (95%) of **7** (TBA) as a yellow solid. An 83-mg sample (22 μmol) in MeCN (1 mL) was treated with a solution of $Cs_2B_{10}Br_{10}$[7] (51 mg, 43 μmol) in 1 mL of MeCN. After 15 min the precipitated Cs salt was separated, washed with MeCN, dried to give 57 mg (78%) of **7** (Cs) as an orange solid. This was ion exchanged to **7** (K) (45 mg), identical by ^1H and ^{31}P NMR to an independently prepared sample.[2]

Oxidation of Keggin Aldehyde 9 (K). To a solution of **9** (K) (70 mg, 22 μmol) in 2 mL of water was added Jones reagent (259 mg CrO_3, 0.343 mmol; 1.67 M in CrO_3, 3.0 M in H_2SO_4). After 4 h Me_3NHCl (191 mg, 2.00 mmol) was added. The orange precipitate was collected, washed with water, and ion exchanged to give 51 mg (74%) of **3** (K), identical by ^1H and ^{31}P NMR to an independently prepared sample.[2] The sample was dissolved in 3 mL of MeOH containing 3 drops of H_2SO_4 and 1 mL of benzene and heated at reflux for 1 h. The solvent was evaporated, and the residue was dissolved in 5 mL of water and treated with Me_3NHCl (163 mg). The precipitate was ion exchanged to give 43 mg (84%) of ester **4** (K), the ^1H NMR spectrum of which was identical with independently prepared material.[2]

(Eicosa-μ-oxoundecaoxoundecatungstate)tetra-μ-oxo[μ_{12}-[phosphato(3−)-*O*:*O*:*O*′:*O*′:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴:*O*‴]][(1,2,3,4,5-η)-1-[2-(2,3,5,8-tetrahydro-1,3-dioxo-2-phenyl-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazin-5-yl)ethyl]-2,4-cyclopentadien-1-yl]titanate(4−), Tetrakis(*N*,*N*,*N*-tributyl-1-butanaminium) (14 TBA). To a solution of Keggin HPT diene **13** (TBA) (68 mg, 18 μmol) in 3 mL of MeCN was added 3.7 mg (18 μmol) of Ph–TAD in 0.75 mL of MeCN. Evaporation of the solvent gave 72 mg (100%) of **14** (TBA) as a yellow solid. The 360-MHz ^1H NMR spectrum was identical with that of a sample prepared from **14** (K) (next experiment). Anal. [**14** (TBA)] Calcd for $C_{83}H_{162}N_7O_{41}PTiW_{11}$: C, 24.83; H, 4.07; N, 2.44. Found: C, 24.69; H, 4.03; N, 2.50.

Diels–Alder Adduct 14 (K). A 64.8-mg (21 μmol) sample of **13** (TMA) was ion exchanged to **13** (K). This was dissolved in 2 mL of MeCN and treated with a solution of Ph–TAD (3.7 mg, 21 μmol) in 2 mL of MeCN. Removal of the solvent gave adduct **13** (K) in quantitative yield: ^1H NMR (100 MHz) (CD_3CN) δ 2.0–2.5 (m), 3.10 (t, 2), 3.98 and 4.32 (AB, 2), 4.68 (br s, 1), 6.09 (m, 2), 6.23 and 6.47 (m, 4), 7.48 (br s, 5). The product was dissolved in water (3 mL) and treated with Bu_4NBr (44.4 mg, 0.138 mmol). The precipitate was collected and washed with water to give 82 mg (102%) of **14** (TBA), identical by

NMR to that prepared from **13** (TBA). **14** (TBA): ^{1}H NMR (CD_3CN) δ 0.95 (t), 1.33–1.41 (m), 1.57–1.66 (m), 2.35 (m), 3.05 (t), 3.13 (t), 4.00 and 4.19 (AB, 2), 4.61 (m, 1), 6.00 and 6.23 (AB, 2), 6.18, 6.27, 6.38, and 6.41 (m, 4), 7.37 (pent, 1), 7.46 (d, 4).

Reaction of 13 (TBA) with TAD-Derivatized Silica Gel 15. A solution of **13** (TBA) (37 mg, 9.6 μmol) in 2 mL of MeCN was slurried with 824 mg of the purple silica gel **15**[8,9] (loading, 0.29 mmol TAD/g). The yellow color of the solution was discharged after 5 min. After 48 h the mixture was filtered, and the silica gel was rinsed with MeCN. The combined MeCN solution was evaporated giving 9.0 mg of a colorless oil that exhibited only TBA resonances in the ^{1}H NMR. No ^{31}P resonances were observed, and the characteristic bands of the HPT were absent in the IR spectrum.

In a control experiment, a solution of 99.7 mg of a mixture of **13** (TBA) and $(Bu_4N)_5Ti(=O)PW_{11}O_{39}$[2] (**17**) (ratio, 1.3:1) in 5 mL of MeCN was slurried as above with 400 mg of silica gel **15**. The filtrate gave 46.2 mg of residue (106% recovery based on **17**). The ^{1}H NMR spectrum revealed only TBA resonances, while the ^{31}P revealed a single resonance at −12.7 ppm characteristic of **17**.

[(1,2,3,4,5-η)-1-[4-(1,3-Dioxo-2,3,3a,4,7,7a-hexahydro-2-phenyl-1*H*-isoindol-4-yl)ethyl]-2,4-cyclopentadien-1-yl][octacosa-μ-oxoheneicosaoxo[μ_9-[phosphato(3−)-*O*:*O*:*O*:*O*′:*O*′:*O*″:*O*″:*O*‴:*O*‴]]heptadecatungstate]-tetra-μ-oxo[μ_9-[phosphato(3−)-*O*:*O*:*O*:*O*′:*O*′:*O*″:*O*″:*O*‴:*O*‴]]titanate(7−), Heptakis(*N*,*N*-dimethylmethanamine) (19 TMA) and 19 (K). To a stirred solution of Dawson HPT diene K^+ salt **18** (79.1 mg, 17 μmol) in 1.4 mL of MeCN was added *N*-phenylmaleimide (3.2 mg, 19 μmol). After 5.5 h at 25 °C the solvent was evaporated to give a solid, the fully assigned[12] 360-MHz ^{1}H NMR spectrum of which showed quantitative formation of adduct **19** (K): NMR δ 2.23 (m, 2), 2.34 (d of d, 1), 2.59 (d, 1), 2.61 (d of d, 1), 3.08 and 3.26 (ABX_2, 2), 3.51 (ABX, 1), 3.57 (ABX, 1), 6.03 (m, 2), 6.44–6.64 (m, 4), 7.17 (d, 1), 7.47 (pent, 4); ^{31}P NMR δ −10.08 (s), −13.34 (s). An analytical sample was prepared as the TMA salt **19** (TMA). Anal. Calcd for $C_{42}H_{90}N_8O_{63}P_2TiW_{17}$: C, 10.19; H, 1.83; N, 2.26. Found: C, 10.47; H, 1.83; N, 2.25.

***N*-(4-Aminophenyl)maleimide (26).** To a solution of 1,4-phenylenediamine (1.08 g, 10.0 mmol) in 10 mL of THF was added dropwise over 1 h a solution of maleic anhydride (980 mg, 10.0 mmol) in 2 mL of THF. After 12 h, the precipitate was collected, washed with THF, and dried, giving 1.64 g (80%) of the monomaleamidic acid derivative [mp 163–165 °C (dec): NMR (Me_2SO-d_6) δ 6.27 and 6.45 (AB, 2), 6.53 and 7.28 (AA′BB′, 4)]. A 63-mg (0.306 mmol) sample was dissolved in 5 mL of dry CH_2Cl_2 and treated at 0 °C with 1-hydroxybenzotriazole hydrate (41 mg, 0.30 mmol) followed by dicyclohexylcarbodiimide (70 mg, 0.33 mmol). After 2 h at 0 °C and 12 h at 25 °C the resulting suspension was filtered at 0 °C, and the filtrate was evaporated to dryness. The residue was chromatographed over silica gel (EtOAc–CH_2Cl_2, 1:1), giving 35 mg (63%) of **26** as a red powder [mp 172–173 °C; NMR δ 6.76 and 7.08 (AA′BB′, 4), 6.84 (s, 2)]. A portion was recrystallized from toluene–EtOAc–cyclohexane giving deep red needles, mp 173–174 °C (lit[14] 174–175 °C).

***N*-(4-Isothiocyanatophenyl)maleimide (27).** To a stirred mixture of **26** (30 mg, 0.16 mmol), $CHCl_3$ (0.5 mL), and saturated aqueous $NaHCO_3$ (0.5 mL) was added at 25 °C a solution of thiophosgene (0.02 mL, 30 mg, 0.26 mmol) in 0.2 mL of $CHCl_3$. After 30 min the mixture was extracted with $CHCl_3$. The extract was concentrated and then chromatographed over silica gel. Elution with $CHCl_3$–MeOH, 9:1, gave 22 mg (60%) of **27** as a yellow powder, mp 112–115 °C. Crystallization from 3:1 $CHCl_3$–MeOH gave the analytical specimen as yellow needles: mp 118–119 °C; ^{1}H NMR ($CDCl_3$) δ 6.90 (s, 2), 7.34 and 7.41 (AA′BB′, 4). Anal. Calcd for $C_{11}H_6N_2O_2S$: C, 57.39; H, 2.61; N, 12.17; S, 13.91. Found: C, 57.38; H, 2.54; N, 12.12; S, 13.62.

***N*-(4-Bromoacetamidophenyl)maleimide (29).** To a solution of **26** (29 mg, 0.15 mmol) in 2 mL of CH_2Cl_2 at 0 °C was added pyridine (28 mg, 0.35 mmol) and then a solution of bromoacetyl bromide (47 mg, 0.23 mmol) in 0.5 mL of CH_2Cl_2. After 30 min 20 mL of EtOAc was added, and the mixture was washed with dilute HCl and dried ($MgSO_4$). Evaporation gave 33 mg of crude **29**. This was dissolved in EtOAc and passed through silica gel. Crystallization gave 28 mg (60%) of **29** as a pale yellow powder: mp 234–236 °C (phb); ^{1}H NMR (CD_3CN) δ 3.98 (s, 2), 6.91 (s, 2), 7.30 and 7.70 (AA′BB′, 4); MS, *m*/*e* 307.978 (2) (M^+, calcd for $C_{12}H_9BrN_2O_3$, 307.980), 310 (2), 224 (36), 143 (26), 99 (33), 61 (25), 56 (100). Anal. Calcd for $C_{12}H_9BrN_2O_3$: C, 46.63; H, 2.93; N, 9.06. Found: C, 46.71; H, 2.91; N, 8.85.

Biotinylated Dienophile 30. To a solution of *d*-biotin (41 mg, 0.17 mmol) in 1 mL of DMF at 0 °C was added Et_3N (23 mg, 0.22 mmol) and then methyl chloroformate (25 mg, 0.27 mmol). The suspension was stirred for 30 min, and then **26** (50 mg, 0.26 mmol) and Et_3N (18 mg, 0.18 mmol) in 0.8 mL of DMF were added. The suspension was stirred at 0 °C for 2 h and then concentrated in vacuo to 0.3 mL. It was transferred to a centrifuge tube with 1 mL of MeOH, and then 30 mL of ether was added. The resulting precipitate was isolated, washed with ether and MeCN, and dried to give 54 mg (80%) of crude **30**. A 21-mg sample was dissolved in 0.3 mL of DMF, applied to a 20 × 20 cm preparative TLC plate and dried in vacuo. This was developed in MeOH:MeCN (1:9) to give 14 mg of a pale yellow solid that was dissolved in 1 mL of DMF, filtered through $MgSO_4$, and then treated with 30 mL of ether to give 13 mg (64%) of **30** as a pale yellow powder: mp 247–250 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.30–1.70 (m, 6), 2.33 (t, 2), 2.80 and 2.84 (d of d, 2), 3.12 (m, 1), 4.14 (d of d, 1), 4.30 (d of d, 1), 6.37 and 6.45 (m and m, 2), 7.16 (s, 2), 7.23 and 7.67 (AA′BB′, 4), 10.04 (s, 1); ^{13}C δ 25.02 (C_7), 28.05 and 28.16 (C_8 and C_9), 36.16 (C_{10}), 39.5 (Me_2SO-d_6 and C_6), 55.33 (C_4), 59.16 (C_{6a}), 61.00 (C_{3a}), 119.19, 126.07, 127.22 (Ar), 134.57 (olefin), 138.73 (Ar), 162.64 (C_2), 170.00, and 171.29 (C_{11} and maleimide C=O). Anal. Calcd for $C_{20}H_{22}N_4O_4S\cdot 0.35H_2O$: C, 57.09; H, 5.44; N, 13.31. Found: C, 56.68; H, 5.24; N, 12.95.

3,5-Dimaleimidobenzoic Acid (32). To a suspension of 3,5-diaminobenzoic acid (152 mg, 1.00 mmol) in MeOH (2 mL) was added sodium hydroxide (40 mg, 1.0 mmol). After a few minutes a solution resulted which was treated with a solution of maleic anhydride (980 mg, 10 mmol) in 5 mL of MeOH. After 1 h the precipitate was collected, washed with MeOH and dried, giving 348 mg (94%) of the crude bis maleamidic acid monosodium salt: NMR (Me_2SO-d_6–$CDCl_3$, 1:1) δ 6.22 and 6.46 (AA′BB′, 4), 7.98 (s, 2), 8.37 (s, 1), 10.83 (s, 2). A 370-mg (1.00 mmol) sample was suspended in 40 mL of MeOH and treated with 10 drops of concentrated H_2SO_4. This was stirred at 65 °C for 30 min and the resulting solution was evaporated to dryness. MeCN (15 mL) was added and then removed by evaporation. Then 15 mL of MeCN was added and the mixture was refluxed for 12 h. The resulting white precipitate was separated and washed successively with 5 mL of MeOH, water, and THF, and then dried to give 143 mg (46%) of **32** as a white powder. Recrystallization from Me_2SO–water gave the analytical specimen: mp >300 °C; ^{1}H NMR ($CDCl_3$–Me_2SO-d_6) δ 6.80 (s, 4), 7.54 (t, 1), 7.95 (d, 2). Anal. Calcd for $C_{15}H_8N_2O_6$: C, 57.70; H, 2.56; N, 8.97. Found: C, 57.35; H, 2.31; N, 8.88.

3,5-Dimaleimidobenzoic Acid *N*-Hydroxysuccinimide Ester (33). To a suspension of **32** (31 mg, 0.10 mmol) in dry THF (10 mL) at 0 °C was added *N*-hydroxysuccinimide (12 mg, 0.10 mmol) and a solution of dicyclohexylcarbodiimide (25 mg, 0.12 mmol) in THF (2 mL). The mixture was stirred at 0 °C for 2 h and then at 25 °C for 12 h. The mixture was filtered at 0 °C, and the filtrate was evaporated to dryness, giving 31 mg (79%) of **33** as a white powder. Recrystallization from CH_2Cl_2–ether, EtOAc–hexane, and then CH_2Cl_2–ether gave the analytical sample: mp 170–171 °C (dec); ^{1}H NMR ($CDCl_3$) δ 2.95 (s, 4), 6.95 (s, 4), 7.90 (s, 1), 8.25 (s, 2). Anal. Calcd for $C_{19}H_{11}N_3O_8\cdot 0.5CH_2Cl_2$: C, 51.82; H, 2.65; N, 9.30. Found: C, 52.03; H, 2.50; N, 9.35.

Maleimide-Functionalized Keggin HPT 35. A solution of **34** (29.3 mg, 9.68 μmol) and **28** (8.9 mg, 34 μmol) in 0.25 mL of DMF was stirred at 60 °C for 5 h, and then the solvent was removed in vacuo. The yellow solid was suspended in 1 mL of water and centrifuged to remove a white solid. The solution was evaporated to dryness giving 31.8 mg (100%) of an orange powder. This was dissolved in 1 mL of MeCN and added to 20 mL of ether. The precipitate was washed with ether and dried, giving 15.8 mg of **35** as an orange powder. Extraction of the ether with 1 mL of water and evaporation of the aqueous phase gave an additional 8.5 mg (24% combined yield) of **35**: ^{1}H NMR δ 2.25 (m, 3), 2.58 (m, 2), 3.08 and 3.24 (m, 2), 3.51 (m, 2), 6.03 (m, 2), 6.47–6.72 (m, 4), 6.90 (s, 2), 7.38 (AA′BB′, 4). Anal. Calcd for $C_{25}H_{21}K_4N_2O_{43}PTiW_{11}$: C, 8.45; H, 0.65; N, 0.86. Found: C, 8.66; H, 0.68; N, 0.66.

Bromoacetamide-Functionalized Keggin HPT 36. A solution of **34** (49 mg, 16 μmol) and **29** (5.1 mg, 16 μmol) in 1 mL of CD_3CN was stirred at 60 °C for 7 h. The NMR spectrum showed quantitative formation of **34**. The solution was concentrated (N_2 stream) to 0.2 mL. This was added to 2 mL of ether. The precipitate was washed with ether and dried to give 44 mg (81%) of adduct **36** as an orange powder: ^{1}H NMR δ 2.22 (m, 3), 2.55 (m, 2), 2.98 and 3.22 (m, 2), 3.47 (m, 2), 3.96 (s, 2), 6.00 (m, 2), 6.42–6.68 (m, 4), 7.34 (AA′BB′, 4). Anal. Calcd for $C_{23}H_{22}BrK_4N_2O_{42}PTiW_{11}$: C, 8.28; H, 0.66; N, 0.84; Br, 2.40. Found: C, 8.07; H, 0.72; N, 0.94; Br, 2.07.

***N*-Hydroxysuccinyl Ester-Functionalized Keggin HPT 37.** A solution of **34** (50 mg, 16 μmol) and **31** (5.2 mg, 16 μmol) in 1 mL of CD_3CN was stirred at 60 °C for 7 h and monitored by NMR. Ether (20 mL) was added, and the orange precipitate was collected and dried to give 37.6 mg (69%) of **37** as an orange powder: ^{1}H NMR (CD_3CN) δ 2.32 (m, 2), 2.58 (s, $\simeq$1, hydroxysuccinimide imp), 2.89 (s, $\simeq$3, hydroxysuccinimide ester), 3.07 and 3.24 (m, 2), 3.42 (m, 1), 3.53 (m, 1), 6.04 (m, 2), 6.35 and 6.50 (m, 2), 7.62–8.13 (m). The relative integrals of the NMR spectrum showed that about 25% of the ester had hydrolyzed. Some of

the resonances were obscured by the solvent peak.

Maleimide-Functionalized Dawson HPT 20 (K) and 20 (TMA). A solution of **18** (48 mg, 10 μmol) and **28** (11 mg, 41 μmol) in 0.5 mL of DMF was stirred at 60 °C for 6.5 h, and then 20 mL of ether was added. The precipitate was dried, then suspended in 1 mL of water, and centrifuged to remove a white solid. The aqueous phase was evaporated to dryness to give 54 mg (106%) of **20** (K) as a pale yellow solid which was twice lyophilized from 5 mL of water: ^{1}H NMR 2.26 (m, 3), 2.59 (m, 2), 3.03 and 3.25 (m, 4), 3.52 (m, 2), 6.04 (m, 2), 6.33–6.67 (m, 4), 6.90 (s, 2), 7.37 and 7.44 (AA′BB′, 4). An analytical sample was prepared as **20** (TMA). Anal. Calcd for $C_{46}H_{91}N_9O_{65}P_2TiW_{17}$: C, 10.95; H, 1.82; N, 2.50. Found: C, 11.06; H, 2.01; N, 2.64.

Bromoacetamide-Functionalized Dawson HPT 21 (K). A solution of **18** (47 mg, 10 μmol) and **29** (3.8 mg, 12 μmol) in 0.5 mL of MeCN was stirred at 60 °C. After 30 min a precipitate formed which was redissolved by addition of 5 drops of DMF. After 6 h at 60 °C 20 mL of ether was added. The precipitate was collected, suspended in 1 mL of water, and centrifuged. Evaporation of the aqueous phase gave 47.7 mg (95%) of **21** (K) as a pale orange solid which was twice lyophilized from 5 mL of water: ^{1}H NMR δ 2.05–2.38 (m, 3), 2.58 (m, 2), 3.01 and 3.21 (m, 2), 3.48 (m, 2), 4.00 (s, 2), 5.98 (m, 2), 6.38–6.60 (m, 4), 7.19 and 7.55 (AA′BB′, 4). Anal. Calcd for $C_{23}H_{22}BrK_7N_2O_{64}P_2TiW_{17}$: C, 5.59; H, 0.45; N, 0.57. Found: C, 6.13; H, 0.62; N, 0.69.

Biotin-Functionalized Dawson HPT 22 (K) and 22 (TMA). A solution of **18** (51 mg, 11 μmol) and **30** (4.9 mg, 12 μmol) in 0.4 mL of Me_2SO-d_6 was stirred at 60 °C for 6 h, and then 10 mL of ether was added. The precipitate was washed with ether, dried, then dissolved in 1 mL of water, and lyophilized to give 55 mg (99%) of **22** (K) as a pale orange solid: ^{1}H NMR (D_2O–CD_3CN, 9:1) δ 1.50–1.90 (m, 6), 2.20–2.47 (m, 3), 2.51 (t, 2), 2.66 (m, 2), 2.83 and 3.05 (AB, 2), 3.11 (m, 1), 3.26 and 3.64 (complex AB, 2), 3.40 (m, 2), 4.52 and 4.60 (m, 2), 6.15 (m, 2), 6.52–6.74 (m, 4), 7.30 and 7.76 (AA′BB′, 4). An analytical sample was prepared as **22** (TMA). Anal. Calcd for $C_{52}H_{105}N_{11}O_{65}P_2TiW_{17}$: C, 12.03; H, 2.04; N, 2.97. Found: C, 12.20; H, 1.91; N, 2.75.

"Dimeric" Dawson HPT 23 (K) and 23 (TMA). A solution of Dawson diene **18** (48.165 mg, 10.08 μmol) and 1,4-dimaleimidobenzene (1.365 mg, 5.088 μmol) in 0.5 mL of Me_2SO was stirred at 60 °C for 2 days, and then ether (5 mL) was added. The resulting precipitate was dissolved in 1 mL of water and treated with 10 mg of Me_3NHCl. The precipitated **23** (TMA) was washed with water and then cation exchanged to give 31 mg (65%) of **23** (K): ^{1}H NMR δ 2.25 (m, 6), 2.58 (m, 4), 3.08 and 3.24 (m, 4), 3.51 (m, 4), 6.03 (m, 4), 6.47–6.72 (m, 8), 7.50 (AA′BB′, 4). The analytical sample was prepared by addition of Me_3NHCl to an aqueous solution of **23** (K), giving a precipitate. This was washed with 1:1 acetone–water and dried, giving **23** (TMA) as a pale yellow powder. Anal. Calcd for $C_{78}H_{174}N_{16}O_{126}Ti_2P_4W_{34}$: C, 9.54; H, 1.79; N, 2.28. Found: C, 9.80; H, 1.84; N, 2.07.

Isothiocyanate-Functionalized Dawson HPT 24 (K) and 24 (TMA). In order to remove traces of water prior to the Diels–Alder reaction, a solution of **18** (K) (35 mg, 7.6 μmol) in 1.5 mL of dry MeCN was treated with phenylisothiocyanate (2 mg, 15 μmol) and stirred at 60 °C for 1 h. Then maleimide **27** (4.0 mg, 17 μmol) was added, and the resulting solution was stirred at 60 °C for 6 h. The solvent was removed, and the residue was triturated with $CHCl_3$ (2 × 1 mL) and dried, giving 30 mg (83%) of **24** (K) as a yellow powder [IR (KBr) 2101 cm^{-1}; ^{1}H NMR δ 2.31 (m, 3), 2.69 (m, 2), 3.14–3.37 (m, 2), 3.66 (m, 2), 6.53–6.72 (m, 4), 7.35 and 7.49 (AA′BB′, 4)]. The analytical sample was prepared by adding 10 mg of Me_3NHCl to an aqueous solution of **24** (K). The precipitate was washed with cold water, then dissolved in water, and reprecipitated by the addition of acetone to give **24** (TMA) as a yellow powder. Anal. Calcd for $C_{43}H_{89}N_9O_{63}P_2STiW_{17}$: C, 10.31; H, 1.58; N, 2.52; S, 0.64. Found: C, 9.85; H, 1.52; N, 2.51; S, 0.59.

Carboxylic Acid Functionalized "Dimeric" Dawson HPT 38 (K) and 39 (TMA). A solution of **18** (TMA) (47.775 mg, 10.00 μmol) and bismaleimide **32** (1.561 mg, 5.00 μmol) in Me_2SO (0.2 mL) was stirred at 60 °C for 2 days. Then ether (5 mL) was added, and the precipitated adduct was collected. This was dissolved in water (1 mL) to which was added Me_3NHCl (10 mg). The precipitated TMA salt was washed with water (2 × 0.5 mL) and then cation exchanged to give **38** (K) (35 mg, 73%) as a yellow powder: NMR δ 2.10 (m, 4), 2.30 (m, 2), 2.56 (m, 4), 3.05 and 3.20 (m, 4), 3.55 (m, 4), 6.00 (m, 4), 6.40–6.66 (m, 8), 7.20 (two s likely due to the presence of stereoisomers, 1), 7.70 (s, 2). The analytical sample of **38** (TMA) was prepared from **38** (K). Anal. Calcd for $C_{82}H_{183}N_{17}O_{128}P_4Ti_2W_{34}$: C, 9.92; H, 1.86; N, 2.40. Found: C, 9.94; H, 1.89; N, 2.32.

***N*-Hydroxysuccinimide Ester Functionalized "Dimeric" Dawson HPT 40 (K) and 40 (TMA).** In order to remove traces of water prior to the Diels–Alder reaction, a solution of **18** (K) (24.10 mg, 5.20 μmol) in 0.5 mL of MeCN was treated with 2 mg of phenylisothiocyanate and stirred at 60 °C for 1 h. Then a solution of ester **33** (1.14 mg, 2.78 μmol in 0.2 mL of MeCN was added, and the resulting solution was stirred at 60 °C for 5 h during which time a precipitate appeared. The mixture was evaporated to dryness, and the residue was triturated with $CHCl_3$ and then dried to give 23 mg (91%) of **40** (K) as a yellow powder [NMR (Me_2SO-d_6) δ 2.00–2.50 (m, 16), 2.90 (s, 4), 5.90–6.30 (m, 12), 7.60 (s, 1), 7.95 (s, 2)]. Because the solvent partially obscured the succinimide region, it was not possible to deduce the extent to which hydrolysis had taken place during the Diels–Alder reaction. Elemental analysis of **40** (TMA) (see below) was not sufficiently sensitive to reveal the extent of hydrolysis. Therefore, a 21.5-mg sample of **40** (K) was dissolved in 0.5 mL of 0.5 M phosphate-buffered D_2O (pD 6.8), and the hydrolysis of the ester was monitored by NMR at ambient probe temperature (≃25 °C; *N*-hydroxysuccinyl ester peak partially obscured by other absorptions at 3.03 ppm, *N*-hydroxysuccinimide peak at 2.63 ppm). The first measurement was made within 11 min of mixing and showed that about 25% of the ester had been hydrolyzed. The remaining ester underwent slow hydrolysis.

An analytical sample was prepared from 40 (K) by precipitation of 40 (TMA) which was washed with water (2 × 0.5 mL) and dried. Anal. Calcd for $C_{86}H_{186}N_{18}O_{130}P_4Ti_2W_{34}$: C, 10.00; H, 1.77; N, 2.39. Found: C, 10.08; H. 1.82; N, 2.13.

Reductive Amination of 10 with 6-Aminohexyl Phosphate To Give 41. A solution of **10** (K) (25 mg, 5.0 μmol) and 6-aminohexyl phosphate (10 mg, 51 μmol) in 0.15 mL of 1 M phosphate buffer pH 6.5 at 25 °C was treated for each of 10 days with 0.1 mg of $NaBH_3CN$ (total, 1.0 mg, 16 μmol). Then Me_3NHCl (5 mg, 52 μmol) was added, and the resulting pale pink precipitate was washed with water and then ion exchanged to **41** (K). The ^{1}H NMR spectrum showed the presence of about 7% of alcohol **11** (K). The sample was purified by preparative TLC ($CHCl_3$–MeOH–water, 3:3:1), giving 18 mg (70%) of pure **41** (K): NMR δ 1.25 (br s), 1.50 (m), 1.60 (m) (integral of these 3 peaks, **8**), 2.10 (t, 2), 2.95 (t, 2), 3.10 (t, 2), 3.60 (t, 2), 4.10 (s, 2), 4.30 (t, 2), 6.45 and 6.55 (AA′BB′, 4), 7.10 and 7.45 (AA′BB′, 4); ^{31}P NMR δ −13.35 (s, 1), −10.20 (s, 1), +3.0 (br s, 1). An analytical sample was prepared from purified **41** (K) as **41** (TMA). Anal. Calcd for $C_{45}H_{109}N_9O_{66}P_3TiW_{17}$: C, 10.59; H, 2.15; N, 2.47. Found: C, 10.88; H, 2.11; N, 2.38.

Reductive Amination of 10 with N^6-[[(Aminohexyl)carbamoyl]-methyl]adenosine 5′-Triphosphate (Li Salt) To Give 42. A solution of **10** (K) (20 mg, 4.2 μmol) and N^6-[(6-aminohexyl)carbamoylmethyl]-adenosine 5′-triphosphate (7.0 mg, 11 μmol, Sigma Co.) in 0.1 mL of 1 M phosphate buffer pH 6.5 at 25 °C was treated for each of 10 days with 0.1 mg of $NaBH_3CN$ (total, 1.0 mg, 16 μmol). Then Me_3NHCl (5 mg, 52 μmol) was added, and the resulting precipitate was washed with water and then ion exchanged to **42** (K). The NMR spectrum showed the presence of about 20% of alcohol **11** (K). The sample was purified by preparative TLC ($CHCl_3$–MeOH–water, 3:3:1) and then again subjected to K^+ ion exchange, giving 17 mg (70%) of a 2:1 mixture (see ^{31}P NMR data) of **42** (K) and the corresponding ADP derivative: ^{1}H NMR δ 1.20 (m, 4), 1.50 (m, 4), 2.10 (t, 2), 2.95 (t, 2), 3.35 (m, 4), 4.10 (s, 2), 4.30 (t, 2), 6.00 (m, 2), 6.40–6.55 (AA′BB′, 4), 7.15 and 7.45 (AA′BB′, 4), 8.20 (s, 1), 8.45 (m, 1); ^{31}P NMR δ −5.00 (d, 1), −10.25 (s, 1, HPT), −11.00 (d, 1), −13.80 (s, 1, HPT), −21.00 (t, 1). The relative integral of the overlapping ATP–ADP derived ^{31}P resonances compared with the two HPT resonances indicated that the sample had undergone about 30% hydrolysis to the corresponding ADP HPT. An analytical sample was prepared from preparative TLC-purified **42** (K) as **42** (TMA). There are 7 TMA cations associated with the HPT moiety by elemental analysis, consistent with the behavior of all the other HPTs. The elemental analysis is also consistent with the presence of one H^+ (inner salt) and 3 K^+ cations associated with the ATP segment, a combination consistent with the neutral pH from which precipitation took place. However, the calculated values are not highly sensitive to the ratio of H^+ to K^+ or to the presence of 30% of the ADP derivative. Anal. Calcd for 70% ATP **42**·(7 TMA + 3 K^+ + 1 H^+) + 30% ADP **42**·(7 TMA + 2 K^+ + 1 H^+): C, 11.58; H, 2.05; N, 3.50. Found: C, 11.91 (11.83), H, 2.11 (2.01); N, 3.07 (2.91).

Electron Microscopy. Thin carbon films over holey ones were exposed to glow discharge at a pressure of 100 microns for 30 s. Then 5 μL of a 0.07 mM solution of **10** (K) or **2** (R = H) in distilled water (pH 6.3) was placed on the grid and left for 2 min. Then the excess solution was withdrawn with a filter paper. Electron micrographs were taken on a Philips 420 EM with an ST lens at 40-kV accelerating voltage with a 40-micron objective aperture at magnification of 210 000×.

Acknowledgment. This research was supported by PHS Grant GM 27137 to J.F.W.K. and by RR 01214 to M.B. J.V. received partial support from RR 01777 to Dr. J. Wall. PHS Grant RR02336 and NSF Grant CHE-8411177 provided funds for one of the NMR spectrometers.