molecules contribute to the long-range order of the solid, and that they are perpendicular to the FeOCl layers and oriented along the *b* axis of the crystal (corresponding to the *c* axis of pristine FeOCl).

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Registry No. FeOCl, 13870-10-5; $FeOCl(TTF)_{1/8.5}$, 100858-11-5.

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

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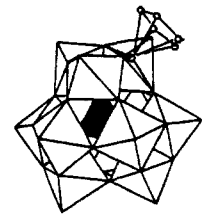
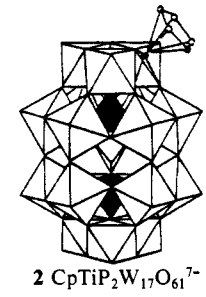
Abstract: With an eye toward the development of a new series of small, highly electron dense labels for electron microscopy, we have synthesized several cyclopentadienyltitanium-substituted Keggin- and Dawson-type heteropolytungstate (HPT) ions that bear reactive organic groups on the Cp ring suitable for selective attachment to macromolecular sites. R-Cp-Ti$(NMe_2)_3$ derivatives **5a-f** were prepared and then inserted into the Keggin defect HPT anion $PW_{11}O_{39}^{7-}$ **7** to give TBA salts **10a-e**. The major byproduct was shown to be oxotitanium compound **14**. Ion exchange of **10a-d** over acidic Al_2O_3 gave the corresponding moderately water soluble TMA salts **11**. Conventional ion exchange then gave the corresponding water soluble K^+ salts **13** and heteropoly acids **12**. Amine **10e** underwent methathetical exchange with $Cs_2B_{10}Br_{10}$ and then ion exchange to give **12e** and **13e**, from which **11e** was prepared. In the Dawson series, a suspension of HPT defect anion **16** in DMF was allowed to react with benzene solutions of **5c,e,f**, giving, after anion exchange on acidic alumina, the corresponding TMA salts **17a-c** which were then converted into K^+ salts **18a-c**. Oxotitanium HPT **21** was the major byproduct. Reaction of **16** with potassium bis(oxalato)oxotitanium(IV) also led to **21**, confirming the structure assignment. The new HPTs were characterized by elemental analysis of their TMA salts and by ^1H, ^{31}P, and ^{183}W NMR spectroscopy on the water soluble K^+ salts.

The direct visualization of cellular ultrastructure by electron microscopy (EM) is now possible routinely at near molecular resolution (<10 Å) and has contributed enormously to an understanding of cellular processes.[2] Central to the EM technique is the introduction of labels[3] of sufficient electron density to be detectable against the background matrix. The iron storage protein ferritin is the most widely used electron dense label. However, the large size of the ferritin-macromolecular complex and the uncertainty in the mode of attachment of the ferritin to the macromolecule limit the resolution to about 200-300 Å. Recognizing the need for new, smaller EM labels, Bartlett et al.[4] introduced the solubilized polycationic undecagold cluster $[(N-H_2C_6H_4)_3P]_7Au_{11}(CN)_2$.[5] Progress has been made toward modification of the cluster for attachment to specific sites of interest[6] and structural results by using these are now becoming available.[7]

The synthesis of cyclopentadienyltitanium-substituted heteropolytungstate (HPT) **1** independently by Klemperer[8] and Knoth[9]



1 $CpTiPW_{11}O_{39}^{4-}$



2 $CpTiP_2W_{17}O_{61}^{7-}$

and the ion's improved hydrolytic stability (up to pH ≃6) over the parent Keggin ion[10] suggested to us a new approach to EM labels, namely, the attachment of reactive organic functional groups to the cyclopentadienyl ring. Dawson-type[11] HPT ions derived from **2** offered the possibility of additional advantages in terms of stability at higher pH values and ease of detection in conventional transmission (CT) EM owing to the presence of 17 tungsten atoms within the ion. Herein, we report the synthesis and characterization of several Cp-substituted Keggin- and Dawson-type HPT ions that bear reactive organic groups suitable for chemoselective covalent bonding to macromolecular sites.[12] The accompanying paper[13] describes further chemical elaboration

(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) Hayat, M. A. *Principles and Techniques of Electron Microscopy. Biological Applications*, 2nd ed.; University Park Press: Baltimore, MD, 1981; Vol. 1.
(3) For a review, see: Hicks, D.; Molday, R. W. In *Science of Biological Specimen Preparation*; Revel, J.-P., Barnard, T., Haggis, G. H., Eds.; Scanning Electron Microscopy, Inc.: AMF O'Hare, IL, 1984; pp 203-219.
(4) Bartlett, P. A.; Bauer, B.; Singer, S. J. *J. Am. Chem. Soc.* **1978**, *100*, 5085.
(5) These clusters have a diameter of about 8.2 Å, with the distance between oppositely situated amino groups being about 25 Å (i.e., total cluster diameter).
(6) Yang, H.; Frey, P. A. *Biochemistry* **1984**, *23*, 3863 and references cited therein.
(7) Kuhn, E.; Fuchs, M.; Varkey, J.; Beer, M. *Proceedings of EMSA Forty-Second Annual Mts.*; Detroit, MI, 1984. Safer, D.; Hainfield, J.; Wall, J. S.; Reardon, J. E. *Science (Washington)* **1982**, *218*, 290.
(8) Ho, R. K. C.; Klemperer, W. G. *J. Am. Chem. Soc.* **1978**, *100*, 6772.
(9) Knoth, W. *J. Am. Chem. Soc.* **1979**, *101*, 759.
(10) Pope, M. T. *Heteropoly and Isopoly Oxometalates*; Springer-Verlag: New York, 1983; p 125.
(11) Dawson, B. *Acta Crystallogr.* **1953**, *6*, 113. D'Amour, V. H. *Acta Crystallogr.; Sect. B: Struct. Crystallogr. Cryst. Chem.* **1976**, *32*, 729.
(12) Lundblad, R. L.; Noyes, C. M. *Chemical Reagents for Protein Modification*; CRC Press: Boca Raton, FL, 1984. Means, G. E.; Feeney, R. E. *Chemical Modification of Proteins*; Holden-Day: San Francisco, CA, 1971.

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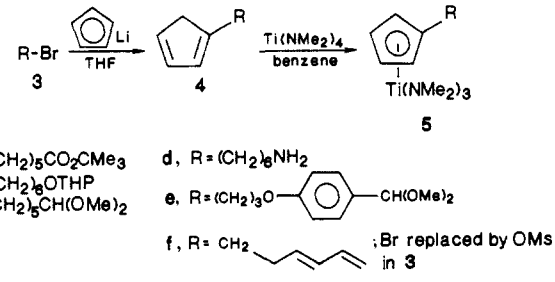
in these series together with CTE micrographs demonstrating the visualization of individual HPT ions.

Reactive organic groups were chosen as targets based on the following considerations. Aldehyde functional groups were sought for eventual reductive amination reactions involving lysine residues. The corresponding alcohols were important as control molecules that are incapable of undergoing the reductive amination reaction. A carboxyl group was sought as a potential acylation agent. Finally, terminal amino groups which might serve to couple the EM label to the macromolecule either via reductive amination, amide formation, or via an alkylation reaction after conversion into the bromoamide were sought.

Preliminary attempts to functionalize directly the cyclopentadienyl ring of **1** or **2** through electrophilic substitution reactions led to degradation of the ions. Therefore, the functionalized alkyl groups were introduced into the Cp ring prior to its attachment (see below) to the HPT ions. Monoalkylation of lithium cyclopentadienide was effected with bromides **3a**,[14] **3b**,[15] **3c**,[16] **3d**,[17] **3e**, and methanesulfonate **3f**, giving respectively, cyclopentadienes **4a–f**.[18] Acid labile protecting groups were chosen since the target

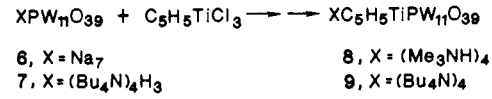


HPTs were expected to be stable to the acidic conditions required for the deprotonation step.

Since Klemperer[8] and Knoth[9] both used $CpTiCl_3$ in the synthesis of **1**, our initial experiments involved metalation of **4b** and subsequent reaction with a excess $TiCl_4$ in hot benzene.[19] While excess $TiCl_4$ was required in order to minimize formation of the bis adduct $(R\text{–}Cp)_2TiCl_2$,[19] the reagent caused a partial cleavage of the THP protecting group with concommitant formation of the corresponding chloride. An alternative method for formation of the η_5-Cp–Ti unit and one compatible with Lewis acid sensitive groups was required.

As part of a study of the reactions of titanium(IV) amides, Lappert[20] reported the preparation of η_5-Cp–$Ti(NMe_2)_3$ from cyclopentadiene and 1 equiv of $Ti(NMe_2)_4$ in high yield. Bis adducts were not formed, presumably owing to the bulky nature of the amino ligands. Application of Lappert's method to cyclopentadienes **4a–f** gave cleanly the moisture sensitive titanium derivatives **5a–c,e,f** in quantitative yield (by NMR). The unprotected amine **5d** could be prepared in a dilute solution; however, an insoluble precipitate is formed upon concentration. Polymerization likely occurred where the amine from one molecule displaced a dimethylamino ligand from another. The ^1H NMR spectra (C_6D_6) of **5a–c,e,f** exhibited a symmetric AA'BB' pattern in the region of δ 6.0 due to the Cp protons, in excellent agreement with the spectrum for Me–Cp–$Ti(NMe_2)_3$ reported by Burger and Dammgen.[21]

Insertion of the functionalized cyclopentadienyl units into a preformed Keggin defect HPT anion $PW_{11}O_{39}^{7-}$ was our next task. In Knoth's[9] preparation of **1**, the Keggin defect ion **6** was allowed



to react with $CpTiCl_3$ in aqueous solution to give the substituted ion, isolated as the trimethylammonium salt **8**. Alternatively, the substitution reaction was done by Klemperer[8] in anhydrous 1,2-dichloroethane by using the tetrabutylammonium salt **7** of the defect HPT and $CpTiCl_3$ to give **9**. Both of these substitution reactions involved the displacement of chloride ligands from the CpTi substrate. Preliminary efforts to replace the dimethylamido ligands with chloride by treatment, for example, of **5a** with a fivefold excess of $TiCl_4$ or **5c** with $SiCl_4$ by using the methodology of Wade and Willey[22] were not encouraging. While some chloride substitution appeared to have taken place (by NMR) in the crude product resulting from the reaction of **5c** with a suspension of anhydrous NH_4Cl in refluxing benzene, a pure product could not be obtained by this method either. A method was therefore needed that would utilize the triamides **5** directly for the Keggin-defect substitution reaction.

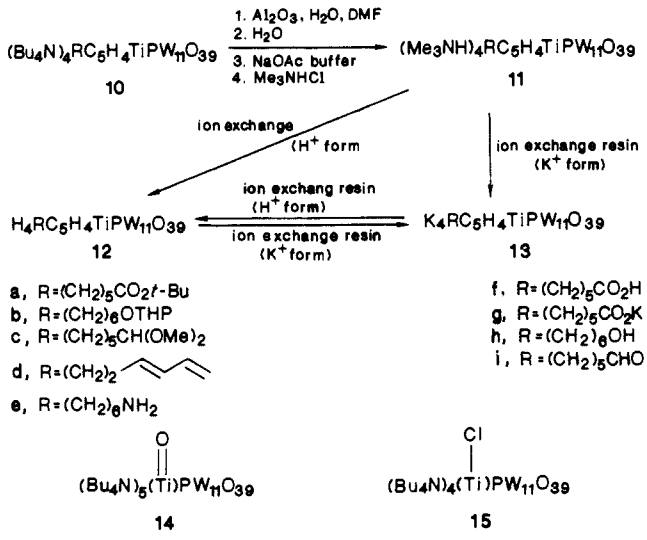
Reactions between **5** and **6** in aqueous solution under a variety of conditions similar to those of Knoth[9] gave little, if any, of the corresponding substituted Keggin derivative. This was likely due to a competing hydrolysis reaction of **5**.[23] The poor aqueous solubility of **5** was another complication which was not improved through use of organic cosolvents.

Methodology similar to that of Klemperer[8] was more fruitful. Treatment of a suspension of **7** in MeCN separately with **5a–d,f** resulted in the corresponding functionalized Keggin tungstate **10**. Conveniently, the three protons of polyoxoanion **7** served to neutralize the 3 equiv of dimethylamide anion generated in the insertion reaction.

In general, purification of the new HPTs herein reported was a nontrivial problem owing to their polar nature. In the case of HPTs **10**, each crude product was deposited onto cellulose powder and then eluted with solvents of increasing polarity. Derivatives **10a–e** could be separated from starting **7** and organic biproducts; however, the titanium oxo compound 14[24] (see below) was always present as a contaminant. Final purification was achieved by chromatography over acidic alumina. Yields of pure **10a–e** were 10–20%, comparable to that for **9**.

The ^1H NMR spectra of Keggin ions **10a–e** showed the expected characteristic AA'BB' pattern in the region of δ 6.2 and 6.4 for the protons on the Cp ring. The ^{31}P NMR spectra consisted of a singlet at δ −13.4, identical with that observed for **9**.[8] The ^{183}W NMR spectra were nearly identical with the six line spectrum of intensity 2:1:2:2:2:2 reported for **9** by Klemperer et al.[25] **10a–e** showed five resonances of intensity 2:3:2:2:2. The chemical shift ($\delta \simeq -94$) of the unique W atom in **10a–e** was coincident with the chemical shift of one of the pairs of W atoms, whereas in **9** two separate peaks (−93.6 (1) and −94.8 (2)) were observed.

(13) Keana, J. F. W.; Ogan, M. D.; Lü, Y.; Varkey, J.; Beer, M. *J. Am. Chem. Soc.*, following paper in this issue.
(14) Castellanos, L.; Gateau-Olesker, A.; Panne-Jacolot, F.; Cleophax, J.; Gero, S. D. *Tetrahedron* **1981**, *37*, 1691.
(15) Mori, K. *Tetrahedron* **1974**, *30*, 3807.
(16) Grotjahn, D. B.; Anderson, N. H. *J. Chem. Soc., Chem. Commun.* **1981**, 306.
(17) Dummont, J. L.; Chodkiewicz, W.; Cadiot, P. *Bull. Soc. Chim. Fr.* **1967**, 588.
(18) Owing to facile 1,5-hydrogen shifts (Andrews, G. D.; Davalt, M.; Baldwin, J. E. *J. Am. Chem. Soc.* **1973**, *95*, 5044) the alkylated cyclopentadienes were obtained as mixtures of double bond isomers in which the two isomers having trisubstituted double bonds predominated (NMR integrations). Each isomer showed a $ABCX_2$ spin system which was essentially identical with the fully analyzed (Mstislavsky, V. I.; Korensvsky, V. A.; Sergeyev, N. M.; Solkan, V. N. *Org. Magn. Reson.* **1976**, *8*, 368) spectrum of methylcyclopentadiene. That a mixture was produced was of little consequence to the synthetic scheme since the next step involved aromatization to a cyclopentadienide ion.
(19) Formation of a cyclopentadienyltitanium bond usually involves the displacement of a chloride ligand: Wailes, P. C.; Coutts, R. S. P.; Wiegold, H. *Organometallic Chemistry of Titanium, Zirconium and Hafnium*; Academic Press, New York, 1974. Bharara, P. C.; Gupta, V. D.; Mehotra, R. C. *J. Organomet. Chem. Libr.* **1977**, *5*, 259–320.
(20) Lappert, M. F.; Chandra, G. *J. Chem. Soc. A* **1968**, 1940.
(21) Burger, H.; Dammgen, U. *J. Organomet. Chem.* **1975**, *101*, 295, 307.
(22) Wade, S. R.; Willey, G. R. *J. Chem. Soc., Dalton Trans.* **1981**, 1264.
(23) The titanium dimethylamides apparently undergo solvolysis more readily than the corresponding chlorides. For example, reaction of $CpTiCl_3$ in hot MeOH gave $CpTi(OMe)Cl_2$ in 77% yield (Gorsich, R. P. *J. Am. Chem. Soc.* **1960**, *82*, 4211) whereas alcoholysis of $CpTi(NMe_2)_3$ gave $Ti(OMe)_4$.[20]
(24) Knoth, W. H.; Domaille, P. J.; Roe, D. C. *Inorg. Chem.* **1983**, *22*, 198.
(25) Klemperer, W. G.; Ho, R. C. K.; Ganzow, O. A. *J. Organomet. Chem.* **1980**, *187*, C27.



The major tungstate byproduct in the preparation of **10a–e** was **14**.[24] The Ti atom in **14** bears a terminal oxo ligand (O^{2-}) instead of a Cp ligand. The structure of **14** was confirmed by two independent preparations. First, **7** was allowed to react with $Ti(NEt_2)_4$ under the conditions used in the preparation of **10**, giving **14**. Second, following Knoth's[24] procedure, $TiCl_4$ was used in place of $Ti(NEt_2)_4$ giving Keggin derivative **15**. The terminal chloride ligand in **15** was then cleaved[24] by using Bu_4NOH to give **14**. The ^{31}P and ^{183}W NMR spectra from both preparations were identical with those of byproduct **14**.

Water soluble analogues of the Keggin salts **10** were required for the envisaged biological applications. Ion exchange in nonaqueous or mixed solvent systems is known to be kinetically slow.[26] Indeed, attempts at cation exchange by using **9** as a model were only partially successful. For example, cation exchange of **9** in MeCN–water (1:1) typically led to mixed salts such as $(Bu_4N)_2Na_2CpTiPW_{11}O_{39}$, as determined by ^1H NMR by using benzene as an internal standard. This mixed salt was not soluble in either water or MeCN but only in mixed solvent systems. Repeated passage through the ion exchange resin resulted in diminished recovery and failed to effect additional ion exchange.

Success was achieved through use of acidic Al_2O_3 which has seen application as an anion exchange medium in aqueous solvent systems.[27] Bu_4N^+ salts **10a–d** were applied in DMF–water, a solvent mixture sufficiently polar to effect ion pair separation. The HPT anions were retained on the Al_2O_3 column throughout a water wash and were subsequently eluted with 1 M NaOAc buffer pH 6.2.[28] Treatment of the buffered eluant with trimethylammonium (TMA) chloride precipitated the HPTs **11a,c,d,h**. *tert*-Butyl ester **11a** was recovered from the alumina with its protecting group intact. The acidic Al_2O_3 had effected complete removal of the THP protecting group of **10b** to give alcohol **11h** directly. Acetal **11c** was contaminated with aldehyde **11i**, indicating that partial hydrolysis had taken place.

The moderately water soluble TMA salts could be readily cation exchanged to give the corresponding K^+ salts **13a,c,d,h**. *tert*-Butyl ester **13a** could be deprotected by using acidic ion exchange resin to give heteropoly acid carboxylic acid **12f**. Attempts to generate aldehyde **12i** from acetal **11c** under similar conditions met with failure, possibly owing to aldol condensation side reactions in the presence of the acidic resin. Acetal **13c**, however, could be deprotected by using aqueous HOAc to give aldehyde **13i**. Ion exchange of TMA salts **11d,h** or K^+ salts **13d,h** over an acidic resin gave the heteropoly acids **12d,h**. Finally, cation exchange of heteropoly acid carboxylic acid **12f** gave the K^+ salt **13g**.

Because amine **10e** could not be eluted readily from the alumina in the first ion exchange step, a metathetical exchange reaction was employed to render the anion water soluble. This was accomplished by mixing MeCN solutions of $Cs_2B_{10}Br_{10}$[29] and amine **10e**. The precipitated[30] cesium HPT salt was collected and then cation exchanged to either K^+ salt **13e** or acid salt **12e**. The TMA salt **11e** was obtained from **13e** by addition of Me_3NHCl.

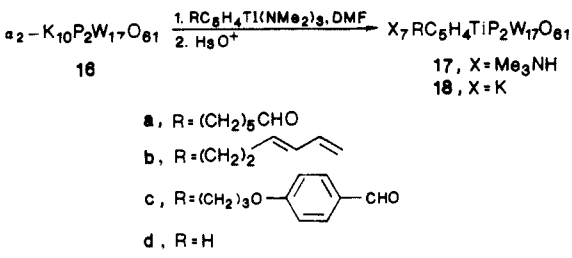
Because, in general, the moderately water soluble TMA HPT salts could be purified conveniently by crystallization from water, elemental analysis data were normally obtained on these salts. After purification, the TMA salts could be readily cation exchanged to give pure samples of the corresponding highly water soluble heteropoly acid or HPT K^+ salt. The water soluble HPTs were characterized by ^1H, ^{31}P, and ^{183}W NMR spectroscopy. The proton spectra in D_2O showed, in addition to the resonances expected for the organic side chains, a characteristic AA'BB' pattern for the Cp ring at about δ 6.5 and 6.7. Despite the presence of the organic residues, water solubility was high, and no evidence of micellar broadening was observed in the proton NMR spectra. The ^{31}P NMR spectra in D_2O all showed a single resonance at about δ −14.0, and the shift was unaffected by the counterion (K^+ or H^+) or pH. The ^{183}W NMR spectra in D_2O–H_2O (1:1) were all similar to that of K^+ salt **13** (R = H) and served to confirm the C_s structure (six resonances in the ratio 2:2:1:2:2:2). For example, **12h** showed resonances (relative integral) at δ −80.44 (2), −104.62 (2), −105.27 (1), −112.77 (2), −123.59 (4, overlapping peaks), while **13i** showed resonances at d −80.02 (2), −104.61 (2), −105.01 (1), −112.40 (2), −123.43 (2), −123.76 (2).

A parallel series of Dawson-type HPTs (see structure **2**) was the next objective. The Dawson defect anion $P_2W_{17}O_{61}^{10-}$ was our starting point. It has been well-characterized[31–34] in the form of the water soluble K, Li, and NH_4 salts, and it has been substituted with several organometallic compounds,[9,35] although these do not include $CpTi^{3+}$.

We envisaged methodology analogous to that used in the synthesis of Keggin derivatives **12** and **13**. However, attempts to prepare and isolate $(Bu_4N)_xH_yP_2W_{17}O_{61}$ were met with failure. Addition of Bu_4NBr to an aqueous solution of the Dawson defect anion did not give a precipitate. Addition of acid to enhance the formation of a mixed salt (cf **7**) gave instead, $(Bu_4N)_6P_2W_{18}O_{62}$, identical by ^{31}P NMR to a sample prepared independently. This result was not totally unexpected since the equilibrium between $P_2W_{17}O_{61}^{10-}$ and $P_2W_{18}O_{62}^{6-}$ is known to be readily established.[10,33,36]

The problem was circumvented with the observation that the substitution reaction can be made to proceed by addition of a benzene solution of Cp–Ti intermediates **5c,e,f** to a suspension of the cap defect isomer, α_2-$K_{10}P_2W_{17}O_{61}$ (**16**)[10,32,33,36] in DMF. Interestingly, the reaction mixture did not assume the characteristic orange color of the substitution products until a subsequent addition of aqueous acid. Thus, apparently it was necessary to protonate the highly basic dimethylamide N atoms at some point during the substitution process. The DMF–water reaction mixtures were subjected to anion exchange on acidic alumina as described in the Keggin series. The substituted Dawson HPTs were eluted with NaOAc buffer, and products **17a–c** were obtained

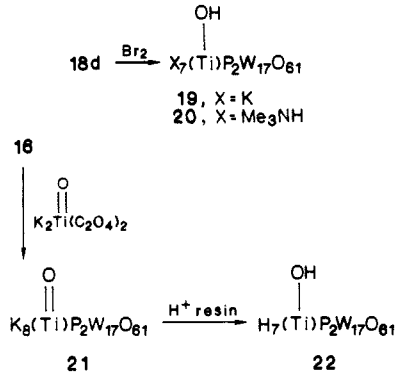
(26) Helfferich, F. *Ion Exchange*; McGraw-Hill: New York, 1962.
(27) Lederer, M.; Polcaro, C. *J. Chromatogr.* **1973**, *84*, 379.
(28) Careful elution permitted the separation of the desired tetravalent Keggin anions from any of the more tightly bound pentavalent oxotitanium contaminant anion (see **14**) that was not removed in the cellulose chromatography step.
(29) We thank Dr. W. H. Knoth for this suggestion and for a generous gift of $Cs_2B_{10}Br_{10}$: Knoth, W. H.; Miller, H. C.; Sauer, J. C.; Balthis, J. H.; Chia, Y. T.; Muetterties, E. L. *Inorg. Chem.* **1964**, *3*, 159.
(30) Pearson's principle of hard and soft acids and bases obtained here. The borane $Cs_2B_{10}Br_{10}$ is a soft base bound to a hard acid cation, and the HPT **10e** is a hard base bound to a soft acid cation. The metathetical exchange produces the hard acid–base pair $Cs_4(RC_5H_4)TiPW_{11}O_{39}$ and the soft acid–base pair $(Bu_4N)_2B_{10}Br_{10}$. See: Pearson, R. G. *Survey of Progress in Chemistry*; **1969**, *5*, 1–52.
(31) Massart, R.; Contant, R.; Fruchart, J.; Ciabrini, J.; Fourier, M. *Inorg. Chem.* **1977**, *16*, 2916.
(32) Acerete, R.; Harmalker, S.; Hammer, C. F.; Pope, M. T.; Baker, L. C. W. *J. Chem. Soc., Chem. Commun.* **1979**, 777.
(33) Contant, R.; Ciabrini, J. *J. Chem. Res. M* **1977**, 2601.
(34) Rocchiccioli-Deltcheff, C.; Thouvenot, R. *Spectrosc. Lett.* **1979**, *12*, 127.
(35) Pope, M. T.; Zonnevijlle, F. *J. Am. Chem. Soc.* **1979**, *101*, 2731.
(36) Souchay, P. *Polycations and Polyanions*; Gauthiers-Villars: Paris, 1963.



by precipitation with TMA·HCl. The oxotitanium impurity **21**[37] (see below) was shown to be present by ^{31}P NMR in the precipitated TMA salts **17a,b**. Repeated crystallization from water gave the pure salts. Ion exchange provided the K^+ salts **18a–c**.

To aid in the NMR spectral characterization of these new Dawson-type HPTs the previously unknown Cp-unsubstituted derivative **18d** was prepared from **16** and $CpTiCl_3$ by using Knoth's[9] procedure. The proton NMR spectra of **18a–d** were indistinguishable from those of the corresponding salts in the Keggin series. The ^{31}P NMR spectra consisted of two singlets in the region of δ −9.93 and −13.19 in D_2O. By way of comparison, the defect anion **16** consisted of two singlets at δ −6.52 and −13.66. Similar upfield shifts of the resonance at δ−6.52 have been observed for products obtained by insertion of transition metals in **16**.[31] The ^{183}W NMR spectra of **18a–d** were all essentially identical, consisting of nine lines in the ratio of 2:2:1:2:2:2:2:2:2. The pattern was consistent with that expected for an α_2 isomer in which the Ti occupies an octahedral site in the cap region giving rise to C_s symmetry. By contrast, if the anions were belt-substituted isomers (α_1),[31-33,36] then the ^{183}W NMR spectra would be expected to exhibit 17 peaks, assuming no coincident resonances.

The structure of oxotitanium impurity **21** was established by additional synthetic work. Treatment of **18d** with aqueous Br_2



led to quantitative cleavage of the Cp–Ti bond. However, the ^{31}P spectrum of the resulting HPT **19** consisted of two singlets at δ −10.32 and −13.14, clearly different from the values δ −9.90 and −13.50 observed for **21**. The nine line ^{183}W NMR spectrum also differed in chemical shift from that of **21**. Combustion analysis of TMA salt **20** (prepared from **19**) indicated that only seven counterions were present, whereas eight were indicated for **21**. Thus, the Br_2 oxidation product was tentatively formulated as the hydroxy titanium HPT **19**.

The structure of **19** was confirmed, and the identity of the oxotitanium impurity as **21** was established as follows. An aqueous solution of **16** in NaOAc buffer pH 5.25 was allowed to react with potassium bis(oxalato)oxotitanium(IV). Addition of Me_3NHCl gave a white precipitate which was ion exchanged to the K^+ salt **21**. The ^{31}P and ^{183}W NMR spectra of this material were identical with those of the impurity formed in the preparation of **17**. Next, a solution of **21** was passed over an acidic ion exchange resin with the expectation that protonation of the oxotitanium group would accompany the cation exchange, leading to HPT acid **22**. The ^{31}P NMR spectrum of **22** was identical with that of K^+ salt **19** obtained from the oxidation of **18d** with Br_2.[38]

(37) Oxotitanium HPT **21** has been mentioned without details: Tourne, C.; Tourne, G. *Bull. Soc. Chim. Fr.* **1969**, 1124.

It is apparent that a variety of organic functionalized Keggin- and Dawson-type HPTs may be prepared. The chemistry of these novel organic–inorganic hybrid anions and their behavior in the electron microscope are described in the accompanying paper.

Experimental Section

NMR spectra were obtained in $CDCl_3$ unless otherwise stated by using either a Varian XL-100 or a Nicolet QE-300 or NT-360 FT spectrometer at ambient temperature. Chemical shift values (δ) are reported relative to an internal sealed capillary of 85% H_3PO_4 for ^{31}P spectra and relative to external 2 M Na_2WO_4 for ^{183}W spectra. For ^1H spectra the residual protons in the deuterated solvents were used as standards: $CHCl_3$, 7.25; Me_2SO, 2.50; DMF, 2.76 and 2.94; water, 4.66; MeCN, 2.95; and benzene, 7.20 ppm. pH measurements were obtained on a Beckman SS-2 or a Corning 125 pH meter. Melting points were measured on a Thomas Hoover apparatus and are uncorrected. Mass spectra were recorded on a CEC 21-110B spectrometer. Elemental analyses were obtained from the University of Oregon Microanalytical Laboratory, Mic Anal Organic Microanalysis, Guelph Chemical Laboratories, Schwarzkopf Microanalytical Laboratory, Galbraith Laboratories, and Mikroanalytisches Labor Pascher.

Preparative TLC was performed with Analtech Inc. 1000 μ silica gel GF plates. Column chromatography utilized either Baker 60–200 mesh or 240–400 mesh silica gel, Woelm acidic aluminum oxide, or Whatman CF-11 cellulose powder.

Tetrahydrofuran (THF) and benzene were distilled from sodium benzophenone ketyl. Ether was distilled from $LiAlH_4$. MeCN and CH_2Cl_2 were distilled from CaH_2. Methanol was distilled from $Mg(OMe)_2$. *N,N*-Dimethylformamide (DMF) was distilled from BaO at 60 °C/40 mm and stored over 4-Å molecular sieves. Ethyl acetate was distilled from K_2CO_3 and stored over 4-Å molecular sieves. Water was doubly distilled. Triethylamine, diisopropylamine, and pyridine were distilled from CaH_2. Reactions were stirred under a N_2 atmosphere that had been pretreated by a column (5 × 45 cm) of BASF F 3-11 copper catalyst followed by a column (2.5 × 48 cm) of 4-Å molecular sieves.

General Procedures for Ion Exchange. Prior to use the cationic exchange resin, Amberlyst 15 W (30 g) was washed with 7 × 100 mL of water, each time decanting the fines, 2 × 100 mL of 95% ethanol, 2 × 100 mL of water, and 4 × 50 mL of 1 M KOH and then rinsed with water to neutrality. The resin was cycled in the column between the K^+ form and the H^+ form as required by using 1 M KOH or 2 M HCl followed by a water rinse to neutrality. Typically, an aqueous solution of the HPT (50 mg in 3 mL of water) was applied to the top of the resin column (1 × 51 cm) and then slowly eluted (1 drop/3–5 s) with water. About 50 mL of eluent was sufficient for total sample recovery. This was rotoevaporated to give the desired cation exchanged HPT (50 mg). In some cases, particularly with TMA salts in the Keggin series, hot (60–70 °C) aqueous solutions (50 mg in 5 mL of water) were applied to the column, and initial elution was done with hot water to ensure solubility of the sample.

General Procedure for Metathetical Exchange to TMA Salts. To a solution of 50–100 mg of the HPT in 10 mL of water contained in a 40-mL centrifuge tube was added Me_3NHCl (100 mg) as a solid. The resulting precipitate was thoroughly mixed with a stirring rod and then centrifuged. The solid was washed and centrifuged twice with a minimal volume (4 mL) of cold water. Analytical samples were obtained by crystallization of the TMA salts from hot water.

1,1-Dimethylethyl 1,3-Cyclopentadiene-1-hexanoate (4a). To a stirred solution of cyclopentadiene (1.115 g, 16.9 mmol) in 20 mL of THF at 0 °C was added 1.3 M *n*-BuLi in hexanes (13.0 mL, 16.9 mmol) dropwise. The resulting white suspension was stirred for 30 min, and then a solution of bromo ester **3a**[14] (1.11 g, 4.43 mmol) in 10 mL of THF was added. After 44 h at 15 °C, 20 mL of water was added. The usual workup[39] with ether gave 1.14 g of an oil which was purified by flash chromatography over silica gel (hexanes–ether, 49:1) to give 599 mg (56%) of **4a** as a colorless oil: NMR δ 1.00–1.80 (m and s, 15), 2.08–2.52 (m and t, 4), 2.88 (d, 2), 5.96–6.58 (m, 3); MS, *m/e* 236.178 (2) (calcd for $C_{15}H_{24}O_2$, 236.178), 180 (15), 91 (22), 57 (100).

2-[[6-(1,3-Cyclopentadien-1-yl)hexyl]oxy]tetrahydro-2*H*-pyran (4b). The preparation was similar to that for **4a** but utilized bromide **3b**[15] (606 mg, 2.29 mmol). There was obtained 344 mg (60%) of **4b** as a slightly yellow oil: NMR δ 1.25–1.97 (m, 14), 1.99 (m, 2), 2.92 (d, 2), 3.28–4.40

(38) We noted above that neither the nature of the counterion (K^+ or H^+) nor the pH had a significant effect on the ^{31}P NMR chemical shifts of HPTs **12** and **13**. Thus, it is reasonable to compare the spectra of **19** and **22**.

(39) The usual workup involved several extractions of the aqueous phase with the indicated organic solvent. The combined extracts were washed with water and saturated NaCl and then dried over $MgSO_4$. Filtration followed by evaporation of the solvent gave the crude product.

(m, 4), 4.61 (m, 1), 6.00–6.47 (m, 3). Anal. Calcd for $C_{16}H_{26}O_2$: C, 76.75; H, 10.47. Found: C, 77.10; H, 10.12.

1-(6,6-Dimethoxyhexyl)-1,3-cyclopentadiene (4c). The preparation was similar to that for **4a** but utilized bromoacetal **3c**[16] (3.971 g, 17.6 mmol). There was obtained 2.05 g (55%) of **4c** as a slightly yellow oil: NMR δ 1.08–1.79 (m, 8), 2.34 (m, 2), 2.87 (d, 2), 3.30 (s, 6), 4.32 (t, 1), 5.88–6.50 (m, 3). Anal. Calcd for $C_{13}H_{22}O_2$: C, 74.24; H, 10.55. Found: C, 74.53; H, 10.36.

1,3-Cyclopentadiene-1-hexanamine (4d). The preparation was similar to that for **4a** but utilized 6-bromo-1-aminohexane hydrobromide[17] and 2 equiv of CpLi. The crude product (382 mg) was purified by silica gel chromatography, eluting with $CHCl_3$–MeOH–NH_4OH (89:10:1) to give 204 mg (37%) of amine **4d** as a yellow oil: NMR δ 1.00–1.80 (m, 10), 2.37 (m, 2), 2.65 (m, 2), 2.89 (d, 2), 5.87–6.53 (m, 3); MS, *m/e* 165.152 (55) (calcd for $C_{11}H_{19}N$, 165.152), 148 (20), 119 (25), 100 (41), 91 (55), 80 (95), 79 (75), 77 (73), 56 (100).

1-(3-Bromopropoxy)-4-(dimethoxymethyl)benzene and 1-[3-(1,3-Cyclopentadien-1-yl)propoxy]-4-(dimethoxymethyl)benzene (4e). A stirred solution of 4-(3-bromopropoxy)benzaldehyde[40] (2.96 g, 12.2 mmol), NH_4NO_3 (209 mg, 2.61 mmol), and trimethylorthoformate (2.85 g, 26.8 mmol) in 15 mL of MeOH was refluxed over 200 mg of $MgSO_4$ for 4 h. The solvent was then removed, and the residue was treated with ether. The suspension was filtered, and the filtrate was evaporated to give 3.49 g of crude acetal. This was combined with 1.61 g of similar material and purified by flash chromatography over silica gel (ether–hexanes, 1:4) to give 3.59 g (70%) of oily product. Crystallization from hexane at 0 °C gave 2.64 g (51%) of the title acetal as colorless needles: mp 33.5–35.5 °C; NMR δ 2.32 (p, 2), 3.34 (s, 6), 3.61 (t, 2), 4.12 (t, 2), 5.36 (s, 1), 6.90 and 7.37 (AX, 4); MS, *m/e* 290.035 (9) (calcd for $C_{12}H_{17}BrO_3$, 290.034), 288 (9), 259 (99), 257 (100).

The preparation of **4e** was similar to that for **4a**. From 1.42 g (4.91 mmol) of the above acetal and 608 mg (9.19 mmol) of cyclopentadiene there was obtained 804 mg (60%) of **4e** as a colorless oil: NMR δ 2.03 (p, 2), 2.56 (m, 2), 2.94 (m, 2), 3.33 (s, 6), 3.99 (t, 2), 5.34 (s, 1), 6.00–6.54 (m, 3), 6.86 and 7.36 (AX, 4); MS, *m/e* 274.157 (1) (calcd for $C_{17}H_{22}O_3$, 274.157), 243 (4), 213 (13), 208 (9), 177 (100).

1-(3,5-Hexadienyl)-1,3-cyclopentadiene (4f). To a stirred solution of (*E*)-3,5-hexadien-1-ol (850 mg, 8.66 mmol)[41] and Et_3N (1.28 g, 12.6 mmol) in 7 mL of CH_2Cl_2 at 0 °C was added dropwise methanesulfonyl chloride (1.49 g, 13.0 mmol). After 30 min the mixture was worked up in the usual way[39] to give 1.60 g of crude oil which was distilled (95 °C, 0.05 mm, bath, 120 °C) to give 1.37 g (90%) of the mesylate **3f** as a colorless oil (*caution*: in scaling up this reaction, attempted distillation of a 14-g sample resulted in an exothermic polymerization believed to be the result of acidic impurities. Therefore, the CH_2Cl_2 solution was washed with aqueous $NaHCO_3$ in later experiments): NMR δ 2.54 (q, 2), 3.02 (s, 3), 4.25 (t, 2), 5.00–6.54 (m, 5).

The preparation of **4f** was similar to that for **4a**. From 770 mg (4.37 mmol) of mesylate **3f** and 480 mg (7.26 mmol) of cyclopentadiene there was obtained a crude oil which was purified by distillation (bath, 60 °C, 0.005 mm) to give 337 mg (53%) of **4f** as a colorless oil: NMR δ 2.43 (m, 4), 2.94 (m, 2), 4.90–6.54 (m, 8). Anal. Calcd for $C_{11}H_{14}$: C, 90.35; H, 9.65. Found: C, 90.11; H, 9.97.

[(1,2,3,4,5-η)-1-[6-(1,1-Dimethylethoxy)-6-oxohexyl]-2,4-cyclopentadien-1-yl]tris(*N*-methylmethanaminato)titanium (5a) and 5b,c,d,e,f.[42] To a solution of $Ti(NMe_2)_4$ (327 mg, 1.46 mmol) in 3.0 mL of benzene at 25 °C was added a solution of **4a** (306 mg, 1.29 mmol) in 2 mL of benzene. The red solution was allowed to stir at 80 °C for 12 h and then used in the next reaction. In one experiment an aliquot was evaporated to dryness to give a viscous red oil. The NMR spectrum showed the reaction to have proceeded quantitatively: NMR (C_6D_6) δ 1.20–1.95 (m) and 1.52 (s) (15), 2.23 (t, 2), 2.56 (t, 2), 3.24 (s, 18), 5.97 (AA′BB′, 4). Benzene solutions of **5b–f** were prepared similarly.

[(1,2,3,4,5-η)-1-[6-(1,1-Dimethylethoxy)-6-oxohexyl]-2,4-cyclopentadien-1-yl](eicosa-μ-oxoundecaoxoundecatungstate)tetra-μ-oxo-[μ_{12}-[phosphato(3–)-*O*:*O*:*O*:*O*′:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴:*O*‴]]titanate(4–), Tetrakis(*N*,*N*,*N*-tributyl-1-butanaminium) (10a). To a stirred suspension of $(Bu_4N)_4H_3PW_{11}O_{39}$ (7)[8] (10.554 g, 2.89 mmol) in 30 mL of MeCN was added a solution of **5a** (1.50 g, 3.60 mmol) in 6 mL of benzene. After 12 h at 25 °C the mixture was evaporated onto cellulose (3 g) and placed on a column of 10 g of cellulose packed in CH_2Cl_2–hexanes 1:1. After an initial elution with this solvent mixture which removed the organic impurities, 7.734 g of crude yellow solid was obtained by elution with 1,2-dichloroethane. The solid was further purified by chromatography over acidic Al_2O_3 (60 g) by using MeCN–H_2O 5:1 as the eluant. The yellow band was collected (3.64 g) and rechromatographed (elution with MeCN–H_2O 24:1) to give 1.57 g (14%) of **10a** as a yellow powder containing a trace of **14** detectible by ^{31}P NMR. The Keggin structures **10b–e** (see below) could be obtained free of **14**, which tended to coelute with the later fractions of the desired salts **10**. Keggin **10a**: ^{31}P NMR (CD_3CN) δ −13.42 (s) and −12.66 (s, <10% impurity of 14); ^1H NMR (CD_3CN) δ 0.98 (t), 1.10–1.84 (m), 1.38 (s), 2.4 (t), 2.88 (t), 3.19 (t), 6.16 and 6.38 (AA′BB′) (integral: CH_2N:Cp = 10:1, calcd 8.5:1); ^{183}W NMR (CD_3CN) δ −76.70 (2), −94.41 (3), −100.53 (2), −112.38 (2), −116.18 (2). Anal. Calcd for $C_{79}H_{167}N_4O_{41}PTiW_{11}$: C, 24.14; H, 4.28; N, 1.43. Found: C, 24.16; H, 4.28; N, 1.59.

Keggin THP Ether 10b.[42] The preparation of **10b** was similar to that for **10a**. From 9.62 g (2.64 mmol) of **7** and 1.37 g (3.18 mmol) of **5b** there was obtained 568 mg (12%) of **10b** as a yellow powder: ^1H NMR (CD_3CN) δ 0.90 (t), 1.14–1.87 (m), 2.92 (t, 2), 3.22 (t, ≃32), 3.30–3.93 (m, 4), 4.51 (s, 1), 6.21 and 6.43 (AA′BB′, 4) (integral: CH_2N:Cp = 11.5:1; calcd 9.5:1); ^{31}P NMR (CD_3CN) δ −13.37 (s); ^{183}W NMR (CD_3CN) δ −76.64 (2), −94.53 (3), −100.64 (2), −112.50 (2), −116.28 (2). Anal. Calcd for $C_{80}H_{169}N_4O_{41}PTiW_{11}$: C, 24.36; H, 4.32; N, 1.42. Found: C, 24.12; H, 4.31; N, 1.48.

Keggin Acetal 10c.[42] The preparation of **10c** was similar to that for **10a**. From 8.12 g (2.22 mmol) of **7** and 849 mg (2.18 mmol) of **5c** there was obtained 1.46 g (17%) of **10c** as a yellow solid: ^1H NMR (CD_3CN) δ 1.00 (t), 1.13–1.87 (m), 2.90 (t, 2), 3.21 (t, 32), 3.26 (s, 6), 4.28 (t, 1), 6.19 and 6.40 (AA′BB′, 4) (integral: CH_2N:Cp = 11.7:1, calcd 10:1); ^{31}P NMR (CD_3CN) δ −13.23 (s); ^{183}W NMR (CD_3CN) δ −76.66 (2), −94.49 (3), −100.59 (2), −112.43 (2), −116.25 (2). Anal. Calcd for $C_{77}H_{165}N_4O_{41}PTiW_{11}$: C, 23.69; H, 4.26; N, 1.44. Found: C, 24.03; H, 4.33; N, 1.50.

Keggin Diene 10d.[42] The preparation of **10d** was similar to that for **10a**. From 7.65 g (2.10 mmol) of **7** and 687 mg (2.11 mmol) of **5f** there was obtained 531 mg (37%) of **10d** as a yellow powder: ^1H NMR (CD_3CN) δ 1.10 (t), 2.20–2.90 (m), 2.58 (m), 3.04 (t, 2), 3.22 (t, 32), 4.88–6.50 (m), 6.26–6.45 (AA′BB′, 4) (integral: CH_2N:olefinic = 3.75:1, calcd 3.78:1); ^{31}P NMR (CD_3CN) δ −13.40 (s); ^{183}W NMR (CD_3CN) δ −77.02 (2), −94.75 (3), −100.74 (2), −112.55 (2), −116.58 (2). Anal. Calcd for $C_{75}H_{157}N_4O_{39}PTiW_{11}$: C, 23.46; H, 4.12; N, 1.46; Ti 1.25; W, 52.66. Found: C, 23.69; H, 3.93; N, 1.34; Ti, 1.01; W, 52.93.

Keggin Amine 10e. The preparation of **10e** was similar to that for **10a**. From 11.16 g (3.06 mmol) of **7** and 1.04 g (3.02 mmol) of **5d** there were obtained from the cellulose chromatography by elution with 1,2-dichloroethane–MeCN, 3:1, yellow fraction A, 2.74 g, and yellow fraction B, 1.70 g. Each was separately rechromatographed twice on acidic Al_2O_3 (20 g) eluting with MeCN–H_2O, 24:1. Fraction A gave 406 mg of amine **10e** having a single ^{31}P resonance at −13.43 ppm: ^1H NMR (CD_3CN) δ 1.00 (t), 1.20–1.95 (m), 2.91 (t, 2), 3.22 (t, 32), 6.22 and 6.43 (AA′BB′, 4) (integral: CH_2N:Cp = 8.4:1, calcd 9.0:1); ^{183}W NMR (CD_3CN) δ −78.56 (2), −94.84 (3), −101.65 (2), −112.51 (2), −116.76 (2). Anal. Calcd for $C_{75}H_{161}N_5O_{39}PTiW_{11}$: C, 23.34; H, 4.23; H, 1.81. Found: C, 21.98; H, 4.09; N, 1.95. This analysis together with the ^1H NMR integral data indicates that the presence of the protonated primary amine group has reduced the number of Bu_4N^+ cations to less than four per Keggin ion. The rechromatography of fraction B gave 1.15 g of a 1:1 mixture of **10e** and $(Bu_4N)_5TiPW_{11}O_{40}$ (**14**): ^{31}P NMR δ −13.79 (s), −13.02 (s).

(Eicosa-μ-oxoundecaoxoundecatungstate)tetra-μ-oxooxo[μ_{12}-[phosphato(3–)-*O*:*O*:*O*:*O*′:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴:*O*‴]]titanate(5–), Pentakis(*N*,*N*,*N*-tributyl-1-butanaminium) (14). To a stirred suspension of **7** (945 mg, 0.259 mmol) in 8.0 mL of MeCN was added $Ti(NEt_2)_4$ (160 mg, 0.48 mmol). After 12 h at 25 °C the mixture was centrifuged, and the supernatant was evaporated to afford 933 mg of a white solid. This was triturated with 2 × 5 mL of hot 1,2-dichloroethane, and the solution was evaporated to afford 160 mg (16%) of **14** as a white powder: ^{31}P NMR (CD_3CN) δ −13.07 (s); ^{183}W (CD_3CN) δ −56.89 (2), −92.03 (2), −101.15 (1), −106.07 (2), −108.58 (2), −117.35 (2). This NMR data was identical with that of **14** prepared by the method of Knoth.[24]

Monohydrogen [(1,2,3,4,5-η)-1-(5-Carboxylatopentyl)-2,4-cyclopentadien-1-yl](eicosa-μ-oxoundecaoxoundecatungstate)tetra-μ-oxo-[μ_{12}-[phosphato(3–)-*O*:*O*:*O*:*O*′:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴:*O*‴]]titanate(5–), Tetrakis(*N*,*N*-dimethylmethanamine) (11f) and 11a, 13a, 12f, and 13g. A mixture containng 776 mg of **10a** and **14** ratio, 6:1 by ^{31}P NMR was dissolved in 2 mL of DMF–H_2O (3:1) and placed on a column of 10 g of acidic alumina packed in the same solvent. Elution with DMF–H_2O gave 346 mg of a $CHCl_3$–soluble oil that showed by NMR only TBA^+ resonances and residual DMF. The column was eluted with 50 mL of

(40) Schweizer, E. E.; Berninger, C. J.; Crouse, D. M.; Davis, R. A.; Logothetis, R. S. *J. Org. Chem.* **1969**, *34*, 207.

(41) Howden, M. E. H.; Maercker, A.; Burdon, J.; Roberts, J. D. *J. Am. Chem. Soc.* **1966**, *88*, 1732.

(42) For a systematic name, see: Ogan, M. D. *Synthesis of Organic Functionalized Cyclopentadienyltitanium Heteropolytungstates Having Keggin and Dawson Structures: Small, Highly Electron Dense, Specific Labels for Electron Microscopy*; Ph.D. Thesis, University of Oregon, 1984. A new nomenclature for polyanions may soon be approved by the IUPAC. See: Jeannin, Y.; Fournier, M., pp 142–146, in ref 10.

H_2O, and then the *tert*-butyl ester was eluted with 70 mL of 1.0 M NaOAc buffer pH 6.2. An aliquot (12 mL) was treated with solid Me_3NHCl (356 mg), and the resulting precipitate, **11a**, was isolated and cation exchanged to afford 57 mg of ester **13a**: ^1H NMR (D_2O) δ 1.38 (s), 1.2–1.83 (m, 15), 2.26 (t, 2), 2.95 (t, 2), 6.50 and 6.67 (AA'BB', 4).

The remaining eluent (58 mL) was subjected directly to sulfonic acid ion exchange resin. The strongly acidic resin cleaved the *tert*-butyl ester to give 294 mg (80%) of carboxylic acid **12f**: ^1H NMR (CD_3CN) δ 1.20–1.90 (m, 6), 2.37 (t, 2), 2.98 (t, 2), 6.54 and 6.80 (AA'BB', 4); ^{31}P NMR (D_2O) δ −14.02 (s); ^{183}W NMR (CD_3CN) δ −80.70 (2), −104.98 (2), −105.57 (1), −112.96 (2), and −123.84 (4) (relative intensities estimated from peak heights).

HPT **12f** was ion exchanged to the K^+ salt **13g**: ^1H NMR (D_2O) δ 1.12–1.92 (m, 6), 2.11 (t, 2), 2.97 (t, 2), 6.54 and 6.69 (AA'BB', 4). A portion of **12f** was subjected to metathetical exchange to give the TMA salt **11f**. Anal. Calcd for $C_{23}H_{55}N_4O_{41}PTiW_{11}$: C, 8.78; H, 1.76; N, 1.78. Found: C, 8.83; H, 1.83; N, 2.07.

(Eicosa-μ-oxoundecaoxoundecatungstate)[(1,2,3,4,5-η)-1-(6-hydroxyhexyl)-2,4-cyclopentadien-1-yl]tetra-μ-oxo[μ_{12}-[phosphato(3−)-*O*:*O*:*O*:*O*′:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴:*O*‴]]titanate(4−), Tetrakis(*N*,*N*-dimethylmethanamine) (11h), 13h, and 12h. A solution of 690 mg of **10b** in 2 mL of DMF–H_2O (4:1) was chromatographed over 15 g of acidic alumina as with **11a**. The NaOAc buffer (75 mL) eluent was treated with Me_3NHCl (1.75 g). The precipitated **11h** was cation exchanged to give 309 mg (61%) of HPT acid alcohol **12h**: ^1H NMR (D_2O) δ 1.00–1.80 (m, 8), 2.80 (t, 2), 3.38 (t, 2), 6.38 and 6.53 (AA'BB', 4); ^{31}P NMR (D_2O) δ −14.03 (s); ^{183}W NMR (D_2O) δ −80.44 (2), −104.62 and −105.27 (3), −112.77 (2), −123.59 (4).

In a separate experiment, ion exchange of crude **11h** gave the K^+ salt **13h** was as an orange solid: ^1H NMR (D_2O) δ 1.10–1.70 (m, 8), 2.90 (t, 2), 3.46 (t, 2), 6.46 and 6.62 (AA'BB', 4). The spectra of **12h** and **13h** showed that the THP group had been cleaved during the purification on alumina.

The analytical sample of TMA salt **11h** was obtained by recrystallization from water. Anal. Calcd for $C_{23}H_{57}N_4O_{40}PTiW_{11}$: C, 8.82; H, 1.84; N, 1.79; Ti, 1.53; W, 64.59. Found: C, 9.00; H, 1.88; N, 2.01; Ti, 1.77; W, 64.67.

HPT Acetals 11c and 13c and Aldehydes 11i and 13i.[42] A solution of 633 mg of **10c** in 2 mL of DMF–H_2O (4:1) was chromatographed over acidic alumina as with **11a**. The NaOAc buffer eluent (75 mL) was treated with Me_3NHCl (1.66 g), and the precipitated **11c** was cation exchanged to give the acetal K^+ salt **13c** (197 mg, 40%) as an orange solid: ^1H NMR (D_2O) δ 1.24–2.00 (m, 8), 3.00 (t, 2), 3.34 (s, 6), 6.56 and 6.72 (AA'BB', 4). Resonances were present corresponding to the presence (25%) of aldehyde **11i** (see below).

A solution of **13c** (117 mg) in 5 mL of water was treated with 15 drops of 1 M HOAc for 3 h. Evaporation gave 115 mg (100%) of aldehyde K^+ salt **13i**: ^1H NMR (D_2O) δ 1.26–2.00 (m, 6), 2.59 (d of t, 2), 3.01 (t, 2), 6.57 and 6.74 (AA'BB', 4) 9.65 (t, 1); ^{31}P NMR (D_2O) δ −13.99 (s); ^{183}W NMR (D_2O) δ −80.02 (2), −104.61 (2), −105.01 (1), −112.40 (2), −123.43 (2), −123.76 (2) (relative intensities estimated from peak height).

An analytical sample of TMA aldehyde **11i** was obtained from **13i** by the general procedure. Anal. Calcd for $C_{23}H_{55}N_4O_{40}PTiW_{11}$: C, 8.83; H, 1.77; N, 1.79. Found: C, 8.89; H, 1.91; N, 2.02.

HPT Dienes 11d and 13d. A solution of 193 mg of **10d** in 3 mL of DMF–H_2O (7:3) was chromatographed over acidic alumina as with **11a**. The NaOAc buffer eluent (50 mL) was treated with Me_3NHCl (358 mg), and the precipitated **11d** (86 mg, 55%) was recrystallized from water to give the analytical sample as an orange solid. Anal. Calcd for $C_{23}H_{53}N_4O_{39}PTiW_{11}$: C, 8.88; H, 1.72; N, 1.80. Found: C, 8.90; H, 1.59; N, 1.70.

TMA salt **11d** was cation exchanged to the K^+ salt **13d** for spectral characterization: ^1H NMR (D_2O) δ 2.57 (q, 2), 3.09 (t, 2), 4.88–6.46 (m, 5), 6.55 and 6.70 (AA'BB', 4); ^{31}P NMR (D_2O) δ −13.95 (s); ^{183}W NMR (D_2O) δ −80.71 (2), −104.92 (2), −105.33 (1), −112.79 (2), −123.38 (2), −123.83 (2).

HPT Amines 11e, 12e, and 13e. The procedure of Knoth[29] was adapted. To a solution of $Cs_2B_{10}Br_{10}$ (279 mg, 0.238 mmol) in 4 mL of MeCN was added a solution of **10e** (342 mg, 0.0886 mmol) in 4 mL of MeCN. After 1 h the precipitate was collected, washed with solvent, and then ion exchanged to give 245 mg (91%) of K^+ salt **13e** as an orange solid: ^1H NMR (D_2O) δ 1.27–2.00 (m, 8), 2.98 (two overlapping t, 4), 6.52 and 6.70 (AA'BB', 4); ^{31}P (D_2O) δ −13.97 (s); ^{183}W NMR (D_2O) δ −80.71 (2), −104.41 (2), −105.48 (1), −112.83 (2), −123.43 (4). Ion exchange on a sulfonic acid resin (see general procedures) gave the HPT acid form **12e**: ^1H NMR (D_2O) δ 1.30–2.00 (m, 8), 3.03 (two overlapping t, 4), 6.55 and 6.72 (AA'BB', 4). HPT **13e** was converted (see general procedure) into the orange microcrystalline TMA salt **11e** for analysis: Anal. Calcd for $C_{23}H_{58}N_5O_{39}PTiW_{11}\cdot 6H_2O$: C, 8.53; H, 2.18; N, 2.16. Found: C, 8.16; H, 1.82; N, 1.94.

[Octacosa-μ-oxoheneicosaoxo[μ_9-[phosphato(3−)-*O*:*O*:*O*:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴]]heptadecatungstate]tetra-μ-oxo-[(1,2,3,4,5-η)-1-(6-oxohexyl)-2,4-cyclopentadien-1-yl][μ_9-[phosphato(3−)-*O*:*O*:*O*:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴]]titanate(7−), Heptakis(*N*,*N*-dimethylmethanamine) (17a) and K^+ Salt 18a. To a suspension of α_2-$K_{10}P_2W_{17}O_{61}\cdot xH_2O$ (**16**) 5.082 g, 1.12 mmol) in 30 mL of DMF was added a solution of acetal **5c** (345 mg, 0.885 mmol) in 3 mL of benzene. The pale yellow suspension was stirred for 1 h, and then the pH was adjusted to pH 3–4 with 10 mL of 2 M HCl. The suspension was centrifuged, and the orange supernatant was diluted with 10 mL of water. This was applied to a column of 15 g of acidic alumina packed in DMF–water, 7:3. The column was eluted with the same solvent (30 mL) followed by water to remove residual DMF. The HPT was then eluted off the column with 50 mL of 1 M NaOAc buffer pH 5.6. The resulting golden solution was treated with a solution of Me_3NHCl (1.804 g, 18.9 mmol) in 5 mL of water. The precipitate was recrystallized from hot water (20 mL) to give 936 mg (22%) of aldehyde HPT TMA salt **17a** as orange microcrystals. Anal. Calcd for $C_{32}H_{85}N_7O_{62}P_2TiW_{17}\cdot 2H_2O$: C, 7.95; H, 1.86; N, 2.03; Ti, 0.99; W, 64.69. Found: C, 8.15; H, 1.83; N, 2.07; Ti, 0.94; W, 63.4.

The TMA salt was ion exchanged to the K^+ salt **18a** (see general procedure) for spectral characterization: ^1H NMR (D_2O) δ 1.30–2.00 (br m, 6), 2.61 (d of t, 2), 2.99 (t, 2), 6.51 and 6.68 (AA'BB', 4), 9.68 (t, 1); ^{31}P NMR (D_2O) δ −9.92 (s), −13.19 (s); ^{183}W NMR (D_2O) δ −92.18 (2), −129.87 (2), −168.96 (1), −175.37 (2), −180.84 (2), −192.39 (2), −195.79 (2), −196.72 (2), −218.99 (2). In similar runs, the ^{31}P NMR spectra revealed the presence of impurity **21** (δ −9.90 (s) and −13.50 (s)). The impurity could be removed by repeated recrystallization of the TMA salt **17a** from water.

Dawson HPT Dienes 17b and 18b. The preparation of **17b** was similar to that for **17a**. From 14.48 g (3.18 mmol) of **16** and 1.031 g (3.17 mmol) of diene **5f** there was obtained, after recrystallization from hot water, 1.336 g (10%) of TMA salt **17b** as orange microcrystals. Anal. Calcd for $C_{32}H_{83}N_7O_{61}P_2TiW_{17}$: C, 8.04; H, 1.75; N, 2.05. Found: C, 7.90; H, 1.70; N, 1.92. This salt was ion exchanged to the K^+ salt (orange solid) for spectral characterization: ^1H NMR (D_2O) δ 2.59 (t, 2), 3.10 (t, 2), 4.90–6.50 (br m, 5), 6.52 and 6.69 (AA'BB', 4); ^{31}P NMR (D_2O) δ −9.93 (s), −13.19 (s); ^{183}W NMR δ −92.29 (2), −130.19 (2), −169.70 (1), −174.68 (2), −180.88 (2), −192.64 (2), −196.05 (2), −197.01 (2), −219.07 (2).

Dawson HPT Benzaldehydes 17c and 18c. The preparation of **17c** was similar to that for **17a**. From 7.505 g (1.65 mmol) of **16** and 721 mg (1.59 mmol) of acetal **5e** there was obtained, after recrystallization from hot water, the TMA salt **17c** as orange microcrystals. Anal. Calcd for $C_{36}H_{85}N_7O_{63}P_2TiW_{17}$: C, 8.90; H, 1.76; N, 2.02; W, 64.32. Found: C, 8.83; H, 1.77; N, 1.98; W, 63.5. This salt was ion exchanged to give 1.059 g (14%) of K^+ salt **18c** as an orange solid: ^1H NMR (D_2O) δ 2.19 (m, 2), 3.13 (br t, 2), 4.19 (br t, 2), 5.45 and 5.65 (AA'BB', 4), 7.13 and 7.93 (AA'XX', 4), 9.76 (s, 1); ^{31}P NMR (D_2O) δ −10.15 (s), −13.40 (s); ^{183}W NMR (D_2O) δ −91.73 (2), −130.08 (2), −168.65 (1), −172.14 (2), −179.37 (2), −191.88 (2), −196.17 (2), −197.16 (2), −218.75 (2).

Dawson Cp–HPTs 17d and 18d. To a solution of **16** (3.369 g, 0.740 mmol) in 15 mL of water at 75 °C was added solid $CpTiCl_3$ (173 mg, 0.790 mmol). The suspension was stirred for 10 min and filtered while hot, and then the filtrate was concentrated to 5 mL and then allowed to stand at 5 °C overnight, giving white crystals of starting **16**. The supernatant solution was evaporated to dryness, giving 3.01 g of crude **18d** as an orange solid. The ^{31}P NMR spectrum revealed clean **18d** (δ −10.10, −13.21) and almost no $P_2W_{17}O_{61}^{10-}$ (δ −6.52, −13.66). Oxo compound **21** was not observed. To an aqueous solution (25 mL) of crude **18d** was added Me_3NHCl (8.751 g, 91.6 mmol), and the resulting precipitate was crystallized from hot water to give 2.018 g (57%) of **17d** as orange crystals. Anal. Calcd for $C_{26}H_{75}N_7O_{61}P_2TiW_{17}$: C, 6.65; H, 1.61; N, 2.09. Found: C, 6.48; H, 1.62; N, 2.01.

A 737-mg sample of **17d** was ion exchanged to give 703 mg (98%) of the highly water soluble K^+ salt **18d** as an orange solid: ^1H NMR (D_2O) δ 6.73 (s); ^{31}P NMR (D_2O) δ −10.10 (s), −13.21 (s); ^{183}W NMR (D_2O) δ −90.70 (2), −129.50 (2), −167.26 (1), −175.85 (2), −180.14 (2), −191.89 (2), −194.26 (2), −195.38 (2), −215.87 (2).

[Octacosa-μ-oxoheneicosaoxo[μ_9-[phosphato(3−)-*O*:*O*:*O*:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴]]heptadecatungstate]tetra-μ-oxohydroxy[μ_9-[phosphato(3−)-*O*:*O*:*O*:*O*′:*O*′:*O*″:*O*″:*O*″:*O*‴:*O*‴]]titanate(7−), Heptakis(*N*,*N*-dimethylmethanamine) (20) and K^+ Salt 19. To a solution of **18d** (220 mg, 0.0484 mmol) in 3 mL of water was added a solution of Br_2 (27.2 mg, 0.172 mmol) in 2 mL of water. After 1 h the solvent was evaporated giving **19** as a white solid: ^1H NMR (D_6 Me_2SO) δ 6.60 (br s, OH); ^{31}P NMR (D_2O) δ −10.32 (s), −13.14 (s); ^{183}W NMR (D_2O) δ −127.17 (2), −130.01 (2), −157.92 (1), −169.69 (2), −175.37 (s), −185.75 (2), −189.81 (2), −190.70 (2), −202.55 (2). A portion was converted to the TMA salt (long needles) by using the general procedure. Anal. Calcd for

$C_{21}H_{71}N_7O_{62}P_2TiW_{17}$: 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).

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

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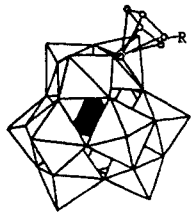
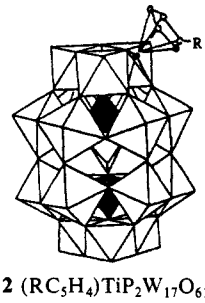
Abstract: Our goal is to develop a series of small, highly electron dense reagents that can be used to label substrate molecules covalently and chemoselectively for subsequent visualization by using conventional transmission electron microscopy (CTEM). Starting with the organic functionalized cyclopentadienyltitanium (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

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