buffer concentration ($k_2[B^-] > k_{-1}$), opening of the intramolecular hydrogen bond is rate-limiting. Values for k_1 and k_{-1}/k_2 were obtained by least-squares analysis of the experimental data in the form of a plot of τ against $1/[B^-]$ as in eq 5, using experimentally determined values of K. The values of k_1 and k_{-1}/k_2 are given in Table I and the curves in Figure 1 were constructed by using these results. The contribution made by hydroxide ion to the observed relaxation time, which would appear as the intercept of a plot of τ^{-1} against buffer concentration at zero buffer concentration, is small and has been neglected in fitting the experimental results to eq 3-5.

The good fit of eq 3-5 to the experimental results supports the proposed mechanism. Further, if it is assumed that proton removal by buffers from the intermediate open forms is diffusion-controlled (k_2 ca. 1×10^{10} dm^3 mol^{-1} s^{-1}) the following values are obtained for opening and closing of the intramolecular hydrogen bonds: for 4-[(4-nitrophenyl)azo]resorcinol monoanion, k_1 77 ± 15 s^{-1} and k_{-1} ca. 7×10^7 s^{-1}, and for 4,6-bis(phenylazo)resorcinol monoanion, k_1 $(3.7 \pm 0.8) \times 10^4$ s^{-1} and k_{-1} ca. 7×10^7 s^{-1}. The applicability of this approach in obtaining similar information about other intramolecularly hydrogen-bonded species is under investigation.

Acknowledgment. The S.E.R.C. and Royal Society are thanked for their support.

Registry No. 4-[(4-Nitrophenyl)azo]resorcinol, 74-39-5; 4,6-bis(phenylazo)resorcinol, 15236-63-2; benzimidazole, 51-17-2; phenol, 108-95-2; 2-methylphenol, 95-48-7; 4-[(4-nitrophenyl)azo]resorcinol monoanion, 63922-66-7; 4,6-bis(phenylazo)resorcinol monoanion, 84174-81-2.

Functionalized Heteropolytungstate Anions Possessing a Modified Dawson Structure: Small, Individually Distinguishable Labels for Conventional Transmission Electron Microscopy

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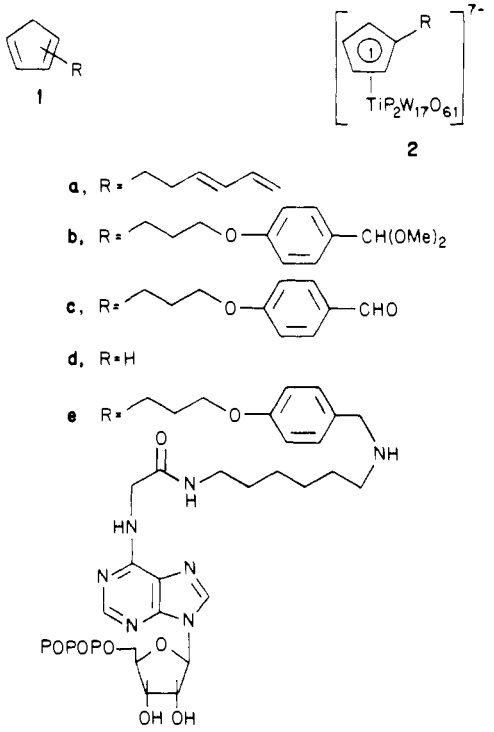
We describe herein the synthesis and properties of a versatile series of Dawson-type[1] heteropolytungstate (HPT) ions[2] that are monofunctionalized with protein-reactive organic groups. Organic transformations effected on the HPTs demonstrate that the HPT unit is stable toward a variety of reaction conditions. The new HPTs contain 17 tungsten atoms within an ellipsoid of about 11 × 18 Å and are shown for the first time to be visible individually using conventional transmission electron microscopy (CTEM),[3,4] constituting a new class of chemoselective labels for CTEM.

The functionalized HPTs were prepared as follows. Alkylation of lithium cyclopentadienide with either 3,5-hexadienyl methanesulfonate[5] or 1-(3-bromopropoxy)-4-(dimethoxymethyl)benzene[6] gave **1a**[7] and **1b**,[8] respectively. Addition[9] of **1a** (309 mg)



to 1.1 equiv of $Ti(NMe_2)_4$ in dry benzene (4 mL) gave a red solution which, after 30 min at 60 °C, was cooled and added to a stirred suspension of 1 equiv of α_2-$K_{10}P_2W_{17}O_{61}$[10] in DMF (20 mL). After 10 min the pale yellow suspension was adjusted to pH 3-4 with 2 M HCl and the resulting orange supernatant was applied to a column of acidic Al_2O_3 packed in 7:3 DMF/water. The orange HPT was eluted with 1 M NaOAc buffer pH 5.6 and precipitated with Me_3NHCl in water. Crystallization from water gave **2a** (TMA salt)[7] (11% yield), which was ion exchanged to the K^+ salt.[11] Benzaldehyde-HPT **2c** (TMA[12] and K^+ salt) was obtained similarly. **2c** was shown to undergo smooth reductive amination with N^6-[[(aminohexyl)carbamoyl]methyl]adenosine 5′-triphosphate (Li salt)[13] to give the HPT-derivatized ATP **2e**, designed for the EM localization of ATP binding sites in certain proteins. HPTs **2a** and **2c** (K^+ salts) are highly water soluble and stable to storage in the solid state or in aqueous solutions (pH 2-8.5) under ambient conditions.

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(2) Pope, M. T. "Heteropoly and Isopoly Oxometalates"; Springer-Verlag: New York, 1983.

(3) The *potential* of HPTs as EM labels has been recognized earlier: Wall, J. S. *Chem. Scr.* **1978**, *14*, 271. Zonnevijlle, F.; Pope, M. T. *J. Am. Chem. Soc.* **1979**, *101*, 273.

(4) Commercially available transmission electron microscopes can provide 2-Å point-to-point resolution with an electron optical magnification of about 800 000. Labels currently in use (for a review, see: Hicks, D.; Molday, R. S. 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) such as ferritin (~120-Å diameter) and colloidal gold (50-1600-Å diameter) are large relative to the resolving power of modern instruments and moreover do not lend themselves well to covalent attachment to biomolecules in a chemically well-defined manner. The recently described cationic undecagold clusters, visible by scanning transmission EM, constitute one approach toward improving labeling methodology through rational design: Reardon, J. E.; Frey, P. A. *Biochemistry* **1984**, *23*, 3849. Yang, H.; Reardon, J. E.; Frey, P. A. *Biochemistry* **1984**, *23*, 3857. Yang, H.; Frey, P. A. *Biochemistry* **1984**, *23*, 3863.

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(7) Satisfactory C, H, and N analytical values were obtained.

(8) MS m/z 274.157 (calcd for $C_{17}H_{22}O_3$, 274.157).

(9) A modification of: Lappert, M. F.; Chandra, G. *J. Chem. Soc. A* **1968**, 1940. In the Keggin series, $(C_5H_5)TiPW_{11}O_{39}^{4-}$ has been prepared independently by using $C_5H_5TiCl_3$: Knoth, W. *J. Am. Chem. Soc.* **1979**, *101*, 759. Ho, R. K. C.; Klemperer, W. G. *J. Am. Chem. Soc.* **1978**, *100*, 6772.

(10) Souchay, P. "Polycations et Polyanions"; Gauthier-Villars: Paris, 1963.

(11) **2a** K^+ salt: ^{1}H NMR (D_2O) 2.59 (t, 2, allylic), 3.10 (t, 2, $CpCH_2$), 4.90-6.50 (m, 5, diene), 6.52, 6.69 ppm (AA′BB′, 4, Cp); ^{31}P NMR (D_2O) -9.93 (s), -13.19 (s) ppm (external H_3PO_4); ^{183}W NMR (D_2O) -92.29 (2 W), -130.19 (2 W), -169.70 (1 W), -174.68 (2 W), -180.88 (2 W), -192.64 (2 W), -196.05 (2 W), -197.01 (2 W), -219.07 ppm (2 W) (external Na_2WO_4).

(12) **2c** TMA salt:[7] calcd % W, 64.32; found, 63.5.

(13) The amine (Sigma Co.) (7 mg), HPT **2c** (20 mg), and $NaBH_3CN$ (0.3 mg, added portionwise) were dissolved in 1 M phosphate buffer pH 6.5 (0.1 mL) and stirred at 25 °C for 10 days. The sample was diluted with water (0.2 mL) and then Me_3NHCl (50 mg) was added to precipitate out the product which was washed with water and ion exchanged to the K^+ salt (20 mg, 88%). 360-MHz NMR showed this to be about 20% of the HPT benzyl alcohol corresponding to **2c**. The entire product was purified by preparative TLC on silica gel, developed with $CHCl_3/CH_3OH/H_2O$, 3:3:1. The middle band was eluted off with water and precipitated as the TMA salt.[7] This was ion exchanged to the K^+ salt of **2e** (11 mg).

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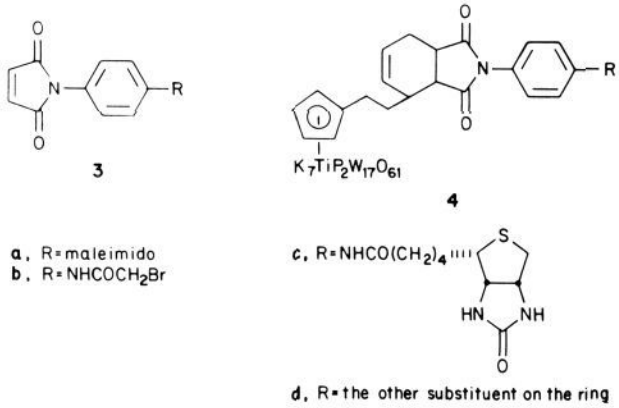
Figure 1. Bright-field TEM of "dimeric" HPT **4d**. The spacing between the dots is about 2–3 nm (different orientations of the dimers) and is consistent with the separation indicated from an inspection of molecular models of an extended conformation of **4d**. The bar represents 20 nm.

The presence of the 1,3-diene unit in HPT **2a** permits the ready attachment of various protein-reactive groups[14] via a Diels–Alder reaction with 4-substituted phenylmaleimide dienophiles. Thus, maleimides **3a–c**[15] were allowed to react (60 °C, 6 h) with 1 equiv



of **2a** (K^+ salt) in DMF, CD_3CN, and Me_2SO-d_6, respectively, giving maleimide **4a**,[16] bromoacetamide **4b**,[16] and the biotinylated derivative **4c**[16] in near quantitative yield. The reaction of 2 equiv of **2a** with **3a** gave the "dimeric" HPT **4d**[16] containing two modified Dawson units.

An electron micrograph (not shown) of HPT **2d** (K^+ salt)[17] taken on a Philips 420 with a ST lens at 40-kV with a 40-μm objective aperture at magnification 210000× consisted of dense dots clearly visible above backgroud. Stability in the beam is high: an exposure taken after 5 min in the beam (beam current giving satisfactory density after 2 s) was indistinguishable from that taken after 2 s. A morphologically unique image consisting of dumbbells results from **4d** (Figure 1), opening the way for differential labeling of multisubunit complexes with these reagents.[18]

Acknowledgment. This research was supported by PHS Grant GM 27137 from the National Institute of General Medical Sciences. We thank Prof. R. Finke for many fruitful discussions.

(14) 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, 1971.

(15) **3a**, Aldrich Co. **3b**, mp 234–236 °C (EtOAc),[7] prepared by acylation of (*p*-aminophenyl)maleimide with bromoacetyl bromide. **3c**·$^1/_3H_2O$, mp 247–250 °C (DMF–ether),[7] prepared by acylation of (*p*-aminophenyl)maleimide with *d*-biotin activated by reaction with methyl chloroformate according to: Green, N. M.; Konieczny, L.; Toms, E. J.; Valentine, R. C. *Biochem. J.* **1971**, *125*, 781.

(16) Formation of the desired adduct was clear from the 360-MHz ^1H NMR spectrum. A satisfactory C, H, and N analysis was obtained on the corresponding TMA salts of **4a–d**.

(17) Prepared by the addition of $C_5H_5TiCl_3$ (173 mg) to an aqueous solution (15 mL) of α_2-$K_{10}P_2W_{17}O_{61}$ (3.368 g)[10] using methodology similar to that of Knoth (see ref 9) in the $PW_{11}O_{39}^{7-}$ series. HPT **2d** (TMA salt);[7] **2d** (K^+ salt); ^1H NMR (D_2O) 6.73 ppm (s); ^{31}P NMR (D_2O) −10.10 (s), −13.21 ppm (s) (external H_3PO_4); ^{183}W NMR (D_2O) −90.70 (2 W), −129.50 (2 W), −167.26 (1 W), −175.85 (2 W), −180.14 (2 W), −191.89 (2 W), −194.26 (2 W), −195.38 (2 W), −215.87 ppm (2 W) (external Na_2WO_4).

(18) Starting with 3,5-dimaleimidobenzoic acid we have also prepared an analogous carboxy-functionalized "dimeric" HPT, characterized as the $(TMA)_{15}$ salt.[7]

Synthesis, Structure, and Reactions of a η^5-CpCo(η^4-bisketene) Complex

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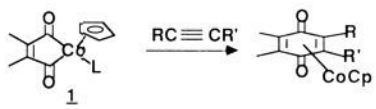
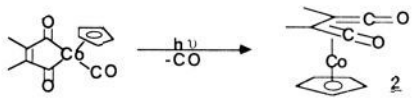
A recent manuscript from this laboratory described the synthesis of η^5-CpCo(dimethylmaleoyl)(L) (**1**) (L = CO, PPh_3, C_5H_5N, Et_2S) and the reaction of certain of these compounds with alkynes to produce a wide range of highly functionalized η^5-CpCo(η^4-1,4-benzoquinone) complexes (eq 1) which can be cleaved to the



(1)

free quinones under mild conditions.[2] It was presumed that dissociation of the ligand L was a necessary prerequisite to reaction with alkynes. In our study of the parent compound **1**, L = CO, the carbon monoxide ligand was found to be very thermally stable but to readily dissociate upon photolysis with a 150-W flood lamp. Photolysis in the presence of an alkyne produced the quinone complex in high yield; however, in the absence of an alkyne a new, reactive cobalt species was produced. Spectroscopic data suggested this new compound was a cobalt complex of a bisketene and an X-ray crystal structure determination confirmed this suspicion. The synthesis, structure, and reactions of this first metal-complexed bisketene are described herein.

Photolysis of 2 mmol of a 0.004 M solution of maleoylcobalt complex **1**, L = CO, in a 1:1 benzene–acetonitrile (nitrogen saturated—with continuous nitrogen purge) for 24 h with a 150-W GE flood lamp placed directly below the reaction vessel led to slow CO evolution accompanied by a color change from bright yellow to deep red. Evaporation of solvent on a rotary evaporator, addition of 50 mL of benzene, brief heating and reevaporation, and repeating the procedure from the addition of benzene gave a residue of crude product. Trituration with hexane yielded 313 mg (66%) of yellow η^5-CpCo(η^4-dimethylbisketene) (**2**) (eq 2).



(2)

An additional quantity of complex (12%) can be obtained from the hexane wash by concentration and chromatography (1:1 Et_2O–hexane; 10 g of flash grade SiO_2).

The dramatic structural reorganization shown in eq 2 was evident from the infrared data (**1**, L = CO, 2025, 1683, 1650 cm^{-1}; **2**, 1810, 1760 cm^{-1}) and NMR spectra (**1**, L = CO, ^1H NMR δ 5.09 (s, 5 H), 1.96 (s, 6 H); ^{13}C NMR δ 240.6, 168.4, 91.8, 12.0; **2**, ^1H NMR δ 5.02 (s, 5 H), 1.91 (s, 6 H); ^{13}C NMR δ 225.9 (CO carbons), 86.8 (olefinic carbons), 51.3 (Cp carbons), 10.8 (CH_3 groups). We could not find one of the ^{13}C resonances of **1**, L = CO, even after extensive pulsing under various conditions. A ^{13}C NMR spectrum of **1**, L = Et_2S (δ 262.0, 166.5, 88.9, 33.2, 13.1, 11.1) confirmed that the very low field signal was due to the acyl carbons and that the CO carbon resonance was missing in the spectrum of **1**, L = CO. Bisketene complex **2** could be crystallized from cold hexane (dec 115 °C) and gave satisfactory elemental analysis. Confirmation of the proposed structure was secured by an X-ray structure determination on a suitable single crystal obtained by slow crystallization from hexane (distilled from CaH_2 under nitrogen). An ORTEP of the molecule is shown in Figure 1 with representative bond distances and angles given in the caption. It is obvious that the cobalt atom lies well below the plane

(1) Fellow of the Alfred P. Sloan Foundation, 1983–1987.

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