as the iodinating species in dediazoniation reactions.

In principle it should be possible to calculate the rate constant, $k_H$, for hydrogen atom abstraction from acetone by **9** or **10** on the assumption that the rate constant for the competing iodine coupling reaction is the same as that, $k_I$, for aryl radicals. In practice the large difference between the magnitudes of $k_H$ and $k_I$ made the outcome subject to very large errors. Our results suggest that $k_H$ at 20 °C is probably in the range $10^4$–$10^5$ $M^{-1}$ $s^{-1}$.

In view of the findings discussed above, it is now possible to define an alternative radical-chain mechanism that is completely consistent with both present and previous experimental observations (Scheme V).

The salient features of the above mechanism are (i) initiation occurs by single electron transfer from iodide ion to the diazonium ion; (ii) the aryl iodine bond is formed by iodine transfer to aryl radical from $I_2$ and/or $I_3^-$ at diffusion controlled rates; and (iii) $I_2^{\bullet-}$, a key chain transfer reagent, is formed by coupling of $I^\bullet$ with $I^-$ and/or iodine atom transfer from $I_3^-$.

## Experimental Section

**General.** Analytical grade acetone was distilled from anhydrous potassium carbonate and stored over 4-Å molecular seives under a nitrogen atmosphere. Sodium iodide was oven dried at 110 °C and stored in a vacuum dessicator. Resublimed iodine (May & Baker) was used without further purification. Potassium ethyl xanthate was prepared by a standard procedure[20] and stored under a nitrogen atmosphere. The preparation of *o*-(but-3-enyloxy)benzenediazonium hexafluorophosphate (**1**) has been described elsewhere.[21] *o*-(But-3-enyloxy)iodobenzene (**4**), 4-(iodomethyl)-3,4-dihydro-2*H*-1-benzopyran (**5**), *o*-(prop-2-ynyloxy)benzenediazonium tetrafluoroborate (**6**), *o*-(prop-2-ynyloxy)iodobenzene (**8**), (*E*/*Z*)-3-(iodomethylene)-2,3-dihydrobenzofuran (**11** and **13**), 3-methylene-2,3-dihydrobenzofuran (**12**), and 3-methylbenzofuran (**14**) were either available from previous studies[8] or were prepared according to procedures previously described.[8]

Gas chromatographic analyses were performed on Varian 3400 and 6000 chromatographs equipped with flame ionization detectors and coupled to Hewlett-Packard 3390A recorder/integrators; 6-ft glass columns packed with either 3% SE-30 on 100/120 mesh Chromosorb W or 2% OV-17 on 60–80-mesh Gaschrom Q, or a 25-m vitreous silica capilliary column (BP1, purchased from SGE Australia), were employed with helium as the carrier gas. GC/MS analyses were carried out on a Varian 1440 gas chromatograph coupled to a VG Micromass 7070F mass spectrometer. Thermostated baths accurate to ±0.3 °C were used for temperature control.

**Mechanistic Studies.** For runs 1–4, Table I, solutions of the diazonium salt (0.1 mmol) in acetone (250 μL) were added to rapidly stirred solutions of NaI/$I_2$ (in the required amounts) in acetone (1.75 mL) with the aid of a microsyringe, the needle-tip of which was held below the surface of the liquid. A darkening of the color and the evolution of gas was usually observed almost instantaneously on mixing of the two solutions. After further stirring under a nitrogen atmosphere for 10–15 min at 20 °C, the solvent was quickly evaporated under vacuum, and a measured amount of hydrocarbon standard was added. The mixture was extracted with ether, washed with 5% sodium thiosulfate solution, dried ($Na_2SO_4$), and analyzed by GC. For run 5, Table I, a solution of potassium ethyl xanthate (0.1 mmol) in acetone (1.0 mL) was added to a solution of the diazonium salt **1** (0.1 mmol) and iodine (0.1 mmol) in acetone (1.0 mL), while for run 6 a solution of sodium iodide (0.1 mmol) and potassium ethyl xanthate (0.1 mmol) in acetone (1.0 mL) was added to a solution of **1** (0.1 mmol) in acetone (1.0 mL).

The results in Table II were obtained by adding 0.1 M solutions (100 μL) of the diazonium salt **1** to solutions (900 μL) of $I_2/I^-$ to achieve the desired concentrations. For each of the runs 1–3, Table III, a solution of the diazonium salt **6** (0.1 mmol) in acetone (250 μL) was added to a solutions of NaI (0.1 mmol) in acetone (0.75, 3.75, and 9.75 mL, respectively), while for run 4 it was added to NaI (0.4 mmol) in acetone (3.75 mL) and for run 5 to a mixture of NaI (0.1 mmol) and $I_2$ (0.02 mmol) in acetone (3.75 mL). Workup and product analyses were carried out as described above.

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# Di-, Tri-, Tetra-, and Pentacationic Alkylammonium Salts. Ligands Designed To Prevent the Nonspecific Electrostatic Precipitation of Polyanionic, Functionalized Cyclopentadienyltitanium-Substituted Heteropolytungstate Electron Microscopy Labels with Cationic Biomolecules

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This work relates to the use of organic functionalized heteropolytungstates (HPTs) as protein-specific electron microscopy labels. The objective is the development of a polycationic ligand that prevents the nonspecific precipitation reaction observed between certain polycationic biomolecules and polyanionic heteropolytungstates. Simple tetra- and pentaammonium salts between Keggin HPT **1b** or Dawson HPT **2b** and amines **3–6** or quaternary ammonium compounds **8** and **9** were all of low aqueous solubility as were salts derived from the oxygenated partners **7**, **11**, **12**, and **15–18**. However, tetrakis quaternary salt **23a**, which is made up of several highly oxygenated alkyl groups, effectively prevents precipitation between Dawson HPT **2b** and the exemplary basic proteins lysozyme and poly-L-lysine as well as the lectin concanavalin A.

Keggin-type and Dawson-type heteropolytungstate (HPT) ions[2] are of interest as possible soluble models for industrially important metal oxide supported catalysts[3] and as small, highly electron dense labels for the study of

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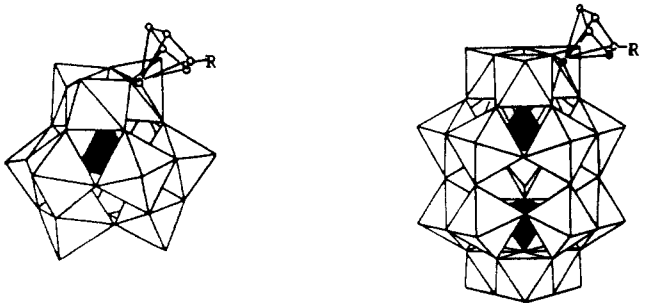
biological specimens by conventional transmission electron microscopy.[4] Our interest in this latter application has resulted in the preparation[4,5] and a study of the reactions[6] of a series of substituted cyclopentadienyltitanium (CpTi) Keggin-type[7] and Dawson-type HPT ions of the general formulas $(\eta_5\text{-}RC_5H_4)TiPW_{11}O_{39}^{4-}$ (1) and $(\eta_5\text{-}RC_5H_4)\text{-}TiP_2W_{17}O_{61}^{7-}$ (2), respectively. The R group represents a chemoselective protein-reactive functional group at the end of an organic spacer arm.



1, $(\eta_5\text{-}RC_5H_4)TiPW_{11}O_{39}^{4-}$ 2, $(\eta_5\text{-}RC_5H_4)TiP_2W_{17}O_{61}^{7-}$

a, R = functionalized organic chain
b, R = H

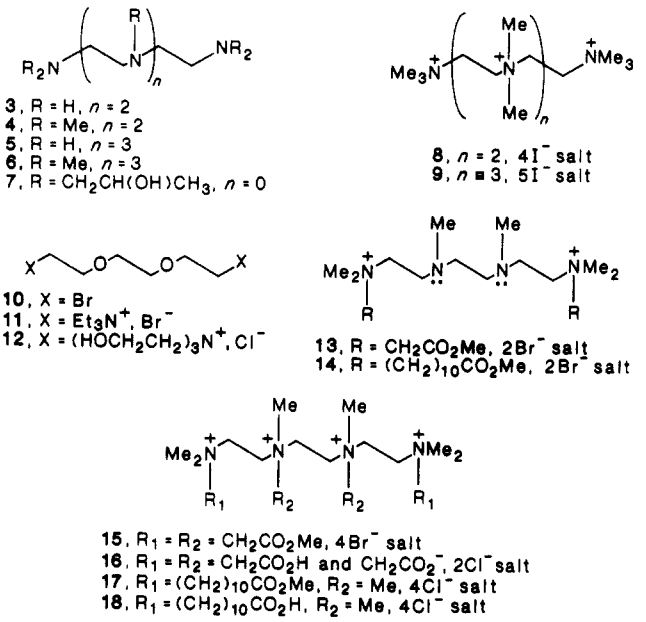
One limitation in the use of these polyanionic HPT EM labels has been a tendency to form insoluble precipitates with biomolecules that bear positively charged groups.[8] This nonspecific electrostatic interaction hampers the intended binding of the HPT to specific sites on the biomolecule by way of the protein-reactive functional group at the end of the spacer arm.

We reasoned that an organic polycation with the charges appropriately spaced and with additional water-solubilizing groups, either ionic or nonionic, might well exhibit preferential electrostatic interaction with the HPTs, thereby effectively preventing precipitation with other cations. Quaternary ammonium groups in particular were eventually chosen owing to their anticipated lack of reactivity under a variety of protein-labeling conditions. Herein, we describe the synthesis of several such polycations and their interaction with the parent HPTs, **1b** and **2b**. We also show that tetrakis(quaternary ammonium) cation **23a** prevents precipitate formation between HPT **2b** and the exemplary basic proteins lysozyme and poly-L-lysine and the lectin concanavalin A under conditions similar to those used for labeling biological systems.

## Results and Discussion

An obvious prerequisite for the success of an alkylammonium ligand for this project was that the ligand itself not form a salt of low aqueous solubility with the HPTs. Thus, the solubility properties of several simple HPT ammonium salts were determined first. Mixing aqueous solutions of Keggin HPT **1b** and the hydrochloride salts of three representative amines, tetraamine **3** (Chart I), pentaamine **5**, and hexane-1,6-diamine, gave an immediate precipitate of the corresponding HPT salt in near quantitative amount. In the case of **5**, the composition of the precipitate was $[H_3N(CH_2CH_2NH_2)_3CH_2CH_2NH_3]_4\text{-}(CpTiPW_{11}O_{39})_5$, indicating that precipitation was preferred over a 1:1 ligand–HPT interaction in which one of the ammonium groups could serve as a water-solubilizing group. Precipitation occurred even in the presence of excess **5**. Quaternary ammonium salts **8** and **9**[9] both gave insoluble salts with **1b**, indicating that the added steric hindrance provided by the extra methyl groups in the quaternary salts did little to inhibit precipitation.

**Chart I**



3, R = H, $n$ = 2
4, R = Me, $n$ = 2
5, R = H, $n$ = 3
6, R = Me, $n$ = 3
7, R = $CH_2CH(OH)CH_3$, $n$ = 0

8, $n$ = 2, $4I^-$ salt
9, $n$ = 3, $5I^-$ salt

10, X = Br
11, X = $Et_3N^+$, $Br^-$
12, X = $(HOCH_2CH_2)_3N^+$, $Cl^-$

13, R = $CH_2CO_2Me$, $2Br^-$ salt
14, R = $(CH_2)_{10}CO_2Me$, $2Br^-$ salt

15, $R_1$ = $R_2$ = $CH_2CO_2Me$, $4Br^-$ salt
16, $R_1$ = $R_2$ = $CH_2CO_2H$ and $CH_2CO_2^-$, $2Cl^-$ salt
17, $R_1$ = $(CH_2)_{10}CO_2Me$, $R_2$ = Me, $4Cl^-$ salt
18, $R_1$ = $(CH_2)_{10}CO_2H$, $R_2$ = Me, $4Cl^-$ salt

Reasoning that the presence of additional oxygen atoms in the ligands might improve the aqueous solubility of their HPT salts, 7·HCl and bis salts **11** and **12** were prepared and then treated with **1b** and/or **2b**. Remarkably, all of these ligands gave either insoluble or only sparingly soluble HPT salts.

Since charged groups tend to be much more effective water-solubilizing groups than nonionic substituents,[10] at this point efforts were turned toward the incorporation of auxiliary charged groups within the polyquaternary ligands. Alkylation of **4** with 2 equiv of methyl bromoacetate gave ester salt **13**, which reacted with excess bromo ester in MeOH at 25 °C to give **15**. Treatment of **15** with silver oxide followed by HCl gave the crystalline salt **16**. Similarly, reaction of **4** with methyl 11-bromoundecanoate gave the heat-sensitive bis quaternary salt **14**, but **14** could not be quaternized further with the bromo ester. Therefore, the two internal nitrogens were quaternized by reaction of **14** with methyl iodide to give tetra salt **17** after ion exchange. This was hydrolyzed to the diacid tetrakis quaternary salt **18** upon treatment with aqueous HCl.

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(7) The parent Keggin HPT **1b** was described independently by Knoth (Knoth, W. *J. Am. Chem. Soc.* **1979**, *101*, 759) and Klemperer (Ho, R. K. C.; Klemperer, W. G. *J. Am. Chem. Soc.* **1978**, *100*, 6772).
(8) **1b**[7] as well as **2b** and the functionalized derivatives **1a** and **2a**[5,6] all rapidly form alkylammonium salts of low aqueous solubility when $Me_3NHCl$ and $Bu_4NCl$ are added to aqueous solutions of the $K^+$ salts. For example, the aqueous solubility of $(Me_3NH)_4CpTiPW_{11}O_{39}$ is about 16 mg/mL at 25 °C.
(9) The commercially available amines were contaminated with other closely boiling amines that could not be satisfactorily removed by fractional distillation. Therefore, it was essential that at some point in a given synthetic scheme, either the polyamine starting material or one of the polyamine intermediates was converted into a crystalline salt that could be purified by repeated recrystallizations. Recovery of the polyamine from the salt then assured isomeric purity in subsequent steps.
(10) Consider that a single ionic group is sufficient to solubilize the sodium dodecyl sulfate detergent molecule in aqueous solution whereas some nine ethyleneoxy units are used to solubilize the nonionic detergent Triton X-100.

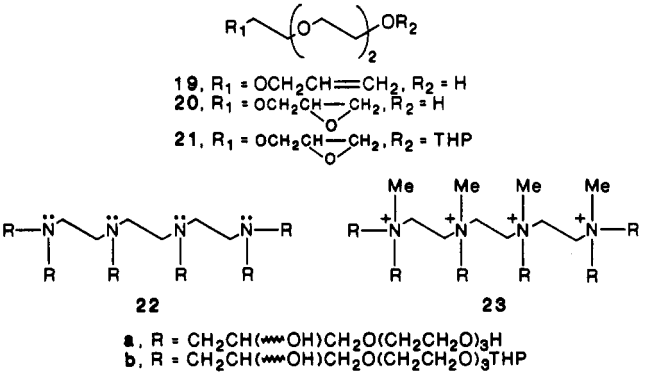
While 18 gave a precipitate with Dawson HPT **2b** over a range of pH, we were encouraged by the observation that tetrakis quaternary salt **16** did not precipitate with **2b** at pH ≥4. Below pH 4 a precipitate formed presumably because the carboxylate anions were protonated, converting the ligand into one similar to tetrakis quaternary ligand 8. The precipitate redissolved upon raising the pH above 4.

The next series of experiments were designed to test operationally the behavior of Dawson HPT **2b** toward a biologically relevant polycationic species in the presence and absence of ligand **16**. Lysozyme[11] is a readily available basic protein (excess of positively charged residues over negatively charged residues at neutral pH, isoelectric point pH 11) of molecular weight 14 388 daltons and was chosen as a relevant model for the interaction of **2b** with other basic proteins of interest for potential EM-labeling experiments such as the histones and other DNA-binding proteins.[12] Lysozyme has 129 amino acid residues, six of which are lysines and eleven of which are arginines. These residues, when taken together with the $NH_2$-terminal group (but ignoring the single histidine residue) amount to a total of 18 positive charges per lysozyme molecule (compared to a total of 11 negatively charged residues). It follows that there are approximately 0.001 mequiv of positive charges per milligram of lysozyme.

Owing to this large number of positively charged residues within its structure, lysozyme was expected to form a precipitate in the presence of **2b**. Indeed, quantitative precipitation was observed (pH ≃6) upon titration of a dilute aqueous solution[13] of lysozyme with 1 negative charge equiv (i.e., the total number of HPT negative charges in the HPT solution equals the total number of lysozyme positive charges in the lysozyme solution) of an aqueous solution of **2b**.

The above protocol was then altered by the addition of 1 charge equiv of ligand **16** to the aqueous solution of **2b** prior to the addition of lysozyme. Unfortunately, quantitative precipitation was observed when lysozyme was added. Only a modest improvement was noted when 8 charge equiv of **16** was used (precipitate yield, 80%).

Success was achieved by utilizing highly oxygenated nonionic solubilizing groups within the polykis quaternary ligand structure. To this end, allyloxy alcohol **19**[14] was oxidized with *m*-chloroperbenzoic acid to give epoxide **20**. The alkylation of amines with epoxides is known[15] to give tertiary amines but not quaternary ammonium compounds. Therefore, tetraamine **3** was allowed to react with an excess of epoxide **20** with the aim of producing tetrakis(tertiary amine) **22a**. The product was then quaternized with excess methyl iodide. While the NMR spectrum indicated that all N atoms had been quaternized, an elemental analysis of the product revealed that seven epoxide units had been incorporated rather than six. Apparently, among the 12 hydroxy groups present in intermediate **22a**, on the average one had undergone alkylation with epoxide **20**.




**a**, R = $CH_2CH(OH)CH_2O(CH_2CH_2O)_3H$
**b**, R = $CH_2CH(OH)CH_2O(CH_2CH_2O)_3THP$

This side reaction could be minimized by protecting the alcohol group of **20**. Thus, **20** was converted into THP ether **21**, which was then used to alkylate **3**, giving tetrakis(tertiary amine) **22b**. Quaternization with MeI gave **23b**, which was hydrolyzed in acid to protective ligand **23a**.

In contrast to the behavior of ligand **16**, ligand **23a** proved to be highly effective in hindering the precipitation reaction between Dawson HPT **2b** and lysozyme. This was demonstrated semiquantitatively as follows. Aqueous solutions (pH 7.1) of lysozyme were separately titrated with aqueous solutions of Dawson HPT **2b** ($K^+$ salt, pH 7.1) both in the absence and presence of protective ligand **23a** in the titrant. Changes in turbidity due to light scattering[16] of the mixtures as a function of added titrant were measured by monitoring the absorbance at 775 nm.[17] Figure 1a shows that precipitation is prevented by the presence of a tenfold charge equivalent excess (approximate lower limite of full effectiveness) of ligand **23a**.

The behavior of two other biologically relevant model polycations, concanavalin A and poly-L-lysine toward **2b** and ligand **23a** was also determined. Concanavalin A was chosen because this lectin, when labeled with the electron-dense EM marker ferritin, has been used for the localization of polysaccharide residues on cell surfaces.[18] The monomeric form of concanavalin A consists of 237 amino acid residues (MW 25 500), 18 of which are either lysines or arginines and 6 of which are histidines. Including the N terminus but ignoring the histidines, there are a total of 19 positively charged residues at pH 7. There are more than balanced by a total of 28 negatively charged residues.[19]

Concanavalin A quantitatively formed a precipitate when titrated with 1 negative charge equiv of **2b**. Possibly the closely spaced positively charged residues between lysine 30 and arginine 60 form a particularly strong association with HPT **2b**. However, when the titration was performed in the presence of 10 positive charge equiv of protective ligand **23a**, no precipitate appeared (Figure 1b).

The strongly basic polypeptide poly-L-lysine provided the most stringent test case. Even in this case precipitation with **2b** could be largely prevented, although a 20-fold positive charge equivalent excess of ligand **23a** was required (Figure 1c).

We conclude that ligand **23a** is effective in enhancing the solubility of Dawson HPT **2b** in the presence of biologically relevant polycations, likely through preferential

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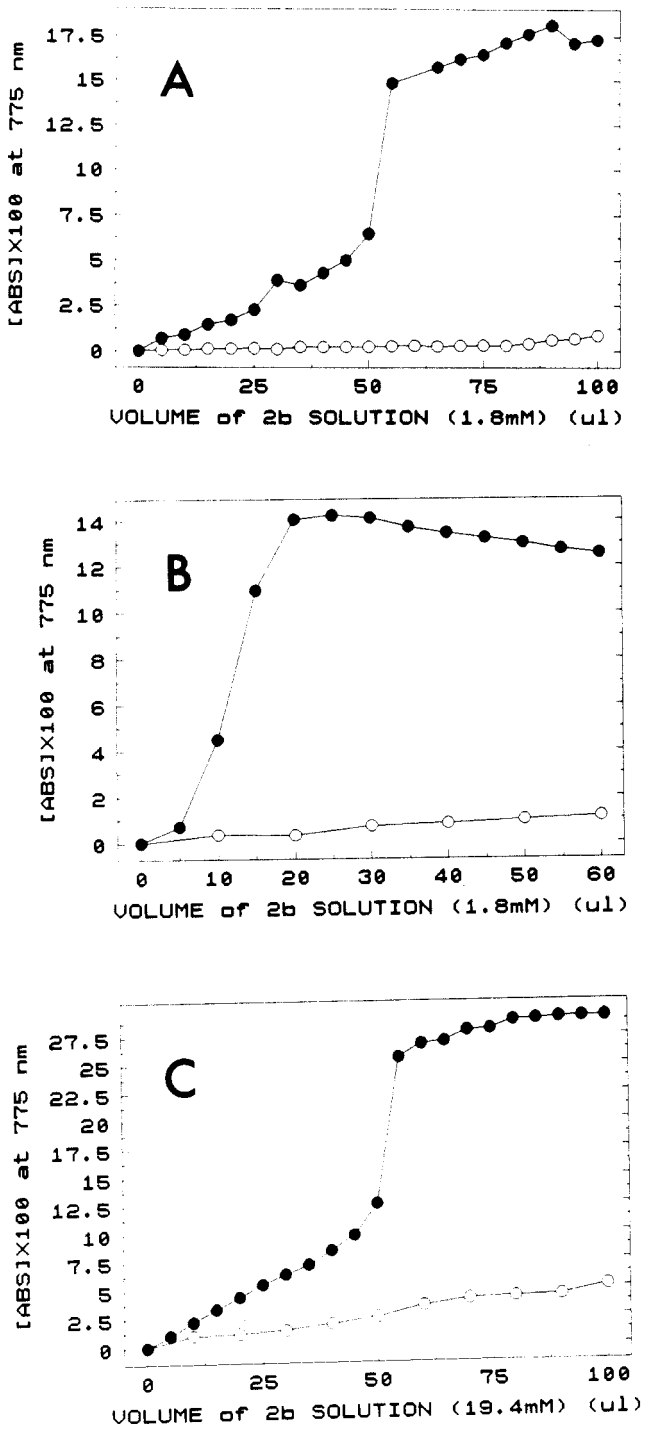


**Figure 1.** Aqueous solutions of (A) lysozyme, (B) concanavalin A, and (C) poly-L-lysine titrated with an aqeous solution of HPT **2b** in the absence (○) and presence (●) of protective ligand **23a**. Increases in turbidity of the mixtures are indicated by increases in absorbance at 775 nm as a function of added titrant.

ion pair formation in solution.

## Experimental Section[20]

**Tetraethylenepentamine Pentahydrochloride (5·5HCl).** To a solution of tetraethylenepentamine (**5**) (5.0 g) in 15 mL of EtOH was added 13 g of concentrated HCl. Filtration gave 9.8 g (100%) of the salt, which was recrystallized from EtOH–water, giving **5·5HCl** (0.85 g), mp 266–268 °C dec. Anal. Calcd for $C_8H_{28}Cl_5N_5$: C, 25.86; H, 7.59; N, 18.84. Found: C, 25.87; H, 7.49; N, 18.59.

***N,N'*-Bis[2-(dimethylamino)ethyl]-*N,N'*-dimethyl-1,2-ethanediamine (4).** An aqueous solution of 88% formic acid (88.4 g, 1.66 mol) was added dropwise to a solution of tetraamine **3** (10.1 g, 0.069 mol, Aldrich) in water (20 mL). Then 37% aqueous formaldehyde (45.0 g, 0.55 mol) was added dropwise, and the mixture was refluxed for 6 h. The reddish-orange solution was cooled, and then concentrated HCl (28 g, 0.13 mol) was added until pH <2. The mixture was concentrated to dryness, and the orange residue was crystallized from MeOH–water, giving 14.2 g (55%) of **4·4HCl** as a white powder, mp 282.5–283.5 °C dec. A solution of 14 g of this salt in 20 mL of water was basified with 10 N NaOH (15 mL) until pH >13. Extraction with benzene gave crude **4**, which was distilled, giving 8.45 g (98%) of pure (by VPC) **4**: bp 74–75 °C (0.05 mm) [lit.[21] bp 110–112 °C (0.2 mm)]; NMR δ 2.21 (s, 12), 2.24 (s, 6), 2.34–2.41 (m, 4), 2.43–2.49 (m, 4), 2.49–2.53 (br s, 4).

Crude **4** was also purified through formation of the tetrahemioxalate salt as follows. A solution of crude **4** (5.79 g, 25.2 mmol) in MeOH (20 mL) was added to a solution of $(COOH)_2{\cdot}2H_2O$ (6.94 g, 55.0 mmol) in MeOH (200 mL). The mixture was heated to the boiling point, and hot water (200 mL) was added until all the solid dissolved. The filtered solution was allowed to cool, and the white precipitate was collected and dried, affording 5.59 g (38%) of a white powder. A 501-mg portion was recrystallized from MeOH–water to give the analytical sample of **4·4($HC_2O_4$)** (365 mg, 73%) as a white powder, mp 238–239 °C. Anal. Calcd for $C_{20}H_{38}N_4O_{16}$: C, 40.68; H, 6.49; H, 9.49. Found: C, 40.61; H, 6.66; N, 9.50.

***N,N,N',N'*-Tetramethyl-*N,N'*-bis[2-(*N,N,N*-trimethylammonio)ethyl]-1,2-ethanediaminium Tetraiodide (8).** A mixture of tetraamine **4** (4.8 g, 21 mmol), MeI (6.5 g, 46 mmol), and 30 mL of EtOH was heated with stirring at 90 °C for 3 h and then allowed to stand overnight. Filtration gave 10.3 g of yellow-white solid, which was partially quaternized **4**. A 4.6-g (20 mmol) sample was combined with 14.2 g (20 mmol) of MeI in 30 mL of EtOH. Water (50 mL) was added, and the solution was refluxed for 2 h. Then 20 mL of EtOH and 5 mL of water were added, and reflux was continued for 3 h. The mixture was allowed to stand overnight at 25 °C. Filtration gave 7.8 g (49%) of white powder. A 1.0-g sample was recrystallized from EtOH–water, giving 690 mg of **8**, mp 198 °C. Anal. Calcd for $C_{16}H_{42}I_4N_4$: C, 24.08; H, 5.30; N, 7.02. Found: C, 24.35; H, 5.17; N, 6.70.

***N,N,N',N'*-Tetramethyl-*N'*-[2-(*N,N,N*-trimethylammonio)ethyl]-*N'*-[2-(*N,N*-dimethyl-*N*-(2-(*N,N,N*-trimethylammonio)ethyl)ammonio)ethyl]-1,2-ethanediaminium Pentaiodide (9).** Salt **9** was prepared from pentaamine **5** via **6** in a manner similar to that of salt **8**. Salt **9** was obtained as a white powder, mp 208–210 °C (EtOH–water). Anal. Calcd for $C_{20}H_{52}I_5N_5$: C, 24.09; H, 5.26; N, 7.02. Found: C, 23.94; H, 5.21; N, 6.76.

**1,2-Bis(2-bromoethoxy)ethane (10).** To a stirred mixture of triethylene glycol (20.0 g, 0.133 mol) and pyridine (4.0 g, 0.51 mol) at 0 °C phosphorus tribromide (28.0 g, 0.103 mol, distilled) was added dropwise over 30 min. The resulting suspension was heated at 60 °C for 4 h, and then the mixture was poured into ice–water (30 mL). The lower organic layer was washed with water containing pyridine (5 × 20 mL), and then it was dried ($MgSO_4$). Vacuum distillation gave a forerun of 1 g of bis(2-bromoethyl) ether [bp 46–50 °C (0.9 mm)], which was followed by pure (VPC) dibromide **10** (19.35 g, 53%, bp 98–100 °C (0.9 mm) [lit[22] bp 95–97 °C (0.3 mm)]): NMR δ 3.48 (t, 4), 3.69 (s, 4), 3.82 (t, 4).

**1,2-Bis[2-(*N,N,N*-triethylammonio)ethoxy]ethane Dibromide (11).** A mixture of **10** (8.3 g, 30 mmol), $Et_3N$ (12 g, 100 mmol), and EtOH (27 mL) was refluxed for 2 h and then more $Et_3N$ (12 g, 120 mmol) and EtOH (27 mL) were added. The mixture was refluxed for 1 day, and then the volatiles were removed, giving 7.15 g (50%) of crude **11**. Crystallization from

(20) Melting points were obtained in a Thoma-Hoover apparatus and are uncorrected. NMR spectra were recorded on either a Varian XL-100 or Nicolet QE 300 spectrometer in $CDCl_3$ unless otherwise stated. Chemical shifts are expressed in δ units with $Me_4Si$ as an internal standard. Elemental analyses were determined by Mic-Anal, Tucson, AZ, or by the Analytical Divison, Academia Sinica, Beijing, PRC.

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acetone–EtOH gave the analytical sample as a dihydrate: mp 91–92 °C; NMR ($D_2O$) δ 1.75 (t, 18), 3.35 (q, 12), 3.50 (t, 4), 3.72 (s, 4), 3.91 (t, 4). Anal. Calcd for $C_{18}H_{42}Br_2N_2O_2 \cdot 2H_2O$: C, 42.02; H, 8.95; N, 5.45. Found: C, 41.77; H, 8.97; N, 5.56. The sample showed mp 150–151 °C when recorded immediately after drying at 56 °C.

***N,N,N′,N′*-Tetrakis(2-hydroxypropyl)ethylenediamine Dihydrochloride (7·2HCl).** A mixture containing 3.47 g (34.2 mmol) of concentrated HCl, 5.00 g (18.0 mmol) of diamine **7** (Aldrich Co.), and 30 mL of EtOH was concentrated and then allowed to stand for 3 days, giving 1.5 g of white crystals. EtOH (15 mL) was added to the filtrate, and the mixture was heated to the boiling point. Water (1 mL) was added to dissolve the suspension. The mixture was allowed to stand at 25 °C for 2 days, giving 1.5 g (combined yield, 46%) of the title salt, mp 185–187 °C. Anal. Calcd for $C_{14}H_{34}Cl_2N_2O_4$: C, 46.03; H, 9.38; N, 7.67. Found: C, 46.04; H, 9.26; N, 7.59.

**1,2-Bis[2-(*N,N,N*-tris(hydroxyethyl)ammonio)ethoxy]-ethane Dichloride (12).** A mixture of dibromide **10** (546 mg, 1.98 mmol) and triethanolamine (1.10 g, 7.37 mmol, obtained from the purified hydrochloride salt, mp 177–178 °C) was heated at 110 °C for 48 h, and then the mixture was extracted with ether in order to remove excess amine. The sticky residue was dissolved in MeOH (0.4 mL) and purified by preparative TLC (MeOH). The middle band gave an oil that was dissolved in 2 mL of water and passed over an ion-exchange column (Amberlite IRA-400, $Cl^-$ form, 1 g), giving 88 mg (8%) of **12** as a colorless hygroscopic oil: NMR δ ($D_2O$) 3.68–3.72 (t, 16), 3.79–3.81 (d, 4), 3.94 (s, 4), 4.02 (s, 10). Anal. Calcd for $C_{18}H_{42}Cl_2N_2O_8 \cdot H_2O$: C, 42.94; H, 8.74; N, 5.57. Found: C, 42.51; H, 8.90; N, 5.52.

***N,N′*-Dimethyl-*N,N′*-bis[2-(*N,N*-dimethyl-*N*-((methoxycarbonyl)methyl)ammonio)ethyl]-1,2-ethanediamine Dibromide (13).** A solution of tetraamine **4** (370 mg, 1.61 mmol) and methyl bromoacetate (983 mg, 6.43 mmol) in MeCN (20 mL) was stirred at 25 °C for 3 h. The precipitate was filtered, washed with MeCN, and dried, giving 819 mg (95%) of crude **13**. Two recrystallizations from MeCN–MeOH gave pure **13** (290 mg, 35%) as a white powder, mp 179–180 °C. Anal. Calcd for $C_{18}H_{40}Br_2N_4O_4$: C, 40.31; H, 7.52; N, 10.46. Found: C, 40.34; H, 7.70; N, 10.36.

***N,N′*-Dimethyl-*N,N′*-bis((methoxycarbonyl)methyl)-*N,N′*-bis[2-(*N,N*-dimethyl-*N*-((methoxycarbonyl)methyl)ammonio)ethyl]-1,2-ethanediaminium Tetrabromide (15).** A solution of **13** (1.32 g, 2.46 mmol) and methyl bromoacetate (1.53 g, 10.0 mmol) in MeOH (4 mL) was heated at 45 °C for 96 h. The resulting white precipitate was collected, washed with MeOH, and dried, giving crude **15** (1.56 g, 75%). Recrystallization from MeOH–water gave pure **15** (351 mg): mp 190–191 °C dec; NMR δ 3.48 (s, 12), 3.49 (s, 6), 3.54 (s, 6), 3.58 (s, 6), 4.06–4.80 (m, 20). Anal. Calcd for $C_{24}H_{50}Br_4N_4O_8$: C, 34.22; H, 5.93; N, 6.65. Found: C, 34.10; H, 6.04; N, 6.63.

***N,N′*-Dimethyl-*N,N′*-bis(carboxymethyl)-*N,N′*-bis[2-(*N,N*-dimethyl-*N*-(carboxymethyl)ammonio)ethyl]-1,2-ethanediaminium Dichloride Dihydrate (16).** A mixture of **15** (69 mg, 82 μmol), $Ag_2O$ (100 mg, 0.43 mmol), and water (2.0 mL) was stirred at 25 °C for 1 h and then filtered. The filtrate was treated with concentrated HCl (0.05 mL) and centrifuged. The supernate was evaporated to dryness, giving 59 mg (84%) of **16** as a white powder. This was dissolved in 1 mL of water and filtered with the aid of a 1-mL water rinse. The aqueous solutions were combined, and the flask was placed inside a large bottle containing acetone (5 mL). The acetone was allowed to diffuse through the atmosphere into the aqueous solution over a two-week period, during which time white needles of **16** formed. After two such recrystallizations there was obtained 32 mg (41%) of pure **16**: mp 157–158 °C dec; NMR δ 3.30 (s, 18), 4.12 (s, 12), 4.26 (m, 8). Anal. Calcd for $C_{20}H_{44}Cl_2N_4O_{10}$: C, 42.03; H, 7.76; N, 9.80. Found: C, 42.48; H, 7.70; N, 9.74.

***N,N,N′,N′*-Tetramethyl-*N,N′*-bis[2-(*N,N*-dimethyl-*N*-(10-(methoxycarbonyl)decyl)ammonio)ethyl]-1,2-ethanediaminium Tetrachloride (17).** A solution of tetraamine **4** (344 mg, 1.50 mmol), methyl 11-bromoundecanoate (1.25 g, 4.48 mmol), and hexane (10 mL) was stirred at 45 °C for 5 h. The white precipitate was filtered, washed with hexane, and dried, giving 593 mg (50%) of crude **14** as a waxy solid: NMR ($CDCl_3$) δ 1.30–1.68 (br s, 32), 2.31 (t, 4), 2.36 (s, 6), 2.72 (m, 4), 3.02 (m, 4), 3.47 (s, 12), 3.90 (m, 4). A solution of crude **14** (593 mg, 0.75 mmol), MeI (430 mg, 3.00 mmol), and MeOH (10 mL) was heated at 45 °C for 6 h, forming a yellow suspension. The mixture was evaporated to dryness, and the residue was dissolved in 2 mL of water. The yellow solution was passed over an ion-exchange column (IRA-400, $Cl^-$ form, 5 g). The colorless eluent was concentrated to dryness (<40 °C), giving **17** (292 mg, 50%) as a white hygroscopic powder. Three recrystallizations from MeCN–MeOH gave the analytical specimen (141 mg, 23%): mp 96–97 °C; NMR δ 1.20 (br s, 32), 2.27 (s, 4), 3.15 (s, 12), 3.90 (s, 6), 4.04 (s, 8), 4.20 (s, 4). Anal. Calcd for $C_{38}H_{82}Cl_4N_4O_4$: C, 56.99; H, 10.22; N, 6.99. Found: C, 56.71; H, 9.73; N, 6.57.

***N,N,N′,N′*-Tetramethyl-*N,N′*-bis[2-(*N,N*-dimethyl-*N*-(10-carboxydecyl)ammonio)ethyl]-1,2-ethanediaminium Tetrachloride (18).** A solution of **17** (379 mg, 0.490 mmol), concentrated HCl (0.02 mL), and water (1.5 mL) was heated at 45 °C for 8 h and then evaporated to dryness. The resulting white powder was crystallized from MeCN–water (9:1, 5 mL), giving **18** (45 mg, 12%) as a white powder: mp 209–210 °C dec; NMR δ 1.27 (br s, 32), 2.33 (t, 4), 3.22 (s, 12), 3.41 (s, 12), 4.28 (s, 8). Anal. Calcd for $C_{36}H_{78}Cl_4N_4O_4 \cdot 2H_2O$: C, 53.46; H, 10.22; N, 6.93. Found: C, 53.77; H, 10.27; N, 6.85.

**2-[2-(2-(2-Propenyloxy)ethoxy)ethoxy]ethanol (19).**[14] 2-[2-(2-Chloroethoxy)ethoxy]ethanol (25.5 g, 0.151 mol) was added dropwise over 30 min at 0 °C to ethyl vinyl ether (33.0 g, 0.46 mol) in the presence of *p*-toluenesulfonic acid·$H_2O$ (20 mg). The mixture was stirred at 15 °C for 1.5 h. More *p*-toluenesulfonic acid·$H_2O$ (15 mg) was added. After 2 h an aqueous solution (2 mL) of $K_2CO_3$ (0.1 g) was added, and the mixture was stirred for 10 min. More $K_2CO_3$ (5 g) was added, and then ammonia was allowed to bubble into the suspension for about 10 min. After filtration and concentration of the filtrate the residue was added dropwise to a solution of sodium allyloxide in allyl alcohol [formed by the reaction of 3.50 g (0.152 mol) of Na with allyl alcohol (50 g)]. The mixture was heated at 100 °C for 8 h and then allowed to cool to 25 °C. Water (50 ml) and aqueous HCl (37%, 5 mL) was added, and the mixture (pH <2) was refluxed at 100 °C for 2 h. Concentration and filtration gave crude **19** which was distilled, giving 22.4 g (78%) of pure (by VPC) **19**: bp 84–86 °C (0.005 mm) [lit.[14] bp 130 °C (3 mm)]; NMR δ 2.82 (br, 1), 3.68 (s, 12), 4.01 (d, 2), 5.12–5.36 (m, 2), 5.74–5.96 (m, 1). Anal. Calcd for $C_9H_{18}O_4$: C, 56.82; H, 9.54. Found: C 56.64; H, 9.73.

**2-[2-(((2-Oxiranylmethoxy)ethoxy)ethoxy)]ethanol (20).** A solution of allyloxy derivative **19** (14.7 g, 77 mmol), 85% *m*-chloroperoxybenzoic acid (16.5 g, 81 mmol), and $CHCl_3$ (30 mL) was heated at 60 °C for 2 h and then cooled to 0 °C. This was filtered, and the filtrate was concentrated to near dryness. Water (20 mL) was added, and the mixture was extracted with ether. The aqueous phase was evaporated to give crude **20**, which was distilled, giving pure **20** (12.1 g, 77%): bp 100–103 °C (0.05 mm); NMR δ 2.48 (br s, 1), 2.68 (t, 1), 2.92 (br s, 1), 3.08 (br s, 1), 3.61 (s, 14). Anal. Calcd for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.09; H, 9.06.

**2-[2-(((2-Oxiranylmethoxy)ethoxy)ethoxy)]ethyl Tetrahydropyranyl Ether (21).** A solution of **20** (7.02 g, 34.0 mmol), dihydropyran (5.92 g, 70.3 mmol), pyridinium *p*-toluenesulfonate (1.7 g, 6.8 mmol), and $CH_2Cl_2$ (80 mL) was stirred for 4 h at 25 °C, and then ether (80 mL) was added. The mixture was extracted with 2.6 M aqueous NaCl and then the organic phase was dried ($MgSO_4$). The solvent was evaporated, and the residue was distilled, giving **21** (6.13 g, 62%) as a colorless oil suitable for the next reaction: bp 126–127 °C (0.05 mm); NMR δ 1.51–1.71 (m, 6), 2.60 (s, 1), 2.78 (s, 1), 3.16 (s, 1), 3.48–3.88 (m, 16), 4.62 (s, 1).

**Preparation of Tetrakis(tertiary amine) 22b and Tetrakis Quaternary Salt 23b.** A neat mixture of epoxide **21** (928 mg, 3.20 mmol) and tetraamine **3** (53 mg, 0.36 mmol) was stirred at 25 °C for 20 days. The resulting crude **22b** was next treated with MeI (0.70 g, 4.9 mmol) in MeOH (3.0 mL) at 42 °C for 24 h, and then the volatiles were evaporated at 25 °C. Preparative TLC (ether) removed excess **21** and left **23b** at the origin. This band was eluted with MeOH (30 mL). The MeOH was evaporated and the residue was dissolved in water–MeOH, 6:1 (2 mL), and passed through an ion-exchange column (Amberlite IRA-400, $Cl^-$, 1 g). The eluent was concentrated to dryness, giving pure **23b** (637 mg, 90%) as a viscous oil suitable for the next reaction. In one run **23b** was obtained as a waxy solid. Anal. Calcd for

$C_{94}H_{180}Cl_4N_4O_{36}\cdot 5H_2O$: C, 51.76; H, 9.06; N, 2.57. Found: C 51.91; H, 9.05; N, 2.73.

**Preparation of Tetrakis Quaternary Ligand 23a.** A solution of **23b** (637 mg) in water (2 mL) containing concentrated HCl (0.1 mL) was stirred at 25 °C for 2 h and then evaporated to dryness. Water (0.5 mL) was added, and the mixture was evaporated to dryness again. The residue was dissolved in MeOH (0.5 mL) and purified by preparative TLC. The plate was developed with ether, it was dried, and then it was redeveloped with MeOH. The band at $R_f$ 0.34 was removed and eluted with MeOH (2 × 0.5 mL). The MeOH was evaporated, and the residue was taken up in water (2 mL) and passed through an ion-exchange column (IRA-400, $Cl^-$ form, 2.5 g). The eluent was evaporated to dryness, and the residue was further dried under vacuum (0.05 mm), giving **23a** (508 mg, 98%) as a yellow, viscous liquid. Anal. Calcd for $C_{64}H_{138}Cl_4N_4O_{30}\cdot 2H_2O$: C, 47.40; H, 8.83; N, 3.46. Found: C, 47.26; H, 8.91; N, 3.51.

**Precipitation Experiments between Ligand 23a and Lysozyme, Concanavalin A, and Polylysine. Lysozyme.** Three millilters (23.3 × 3 × 18 = 1.3 μequiv of positive charge ignoring the single histidine residue) of a stirred 23.3 μM aqueous solution of lysozyme (0.350 mg/mL, pH adjusted to 7.1 by addition of 1 N NaOH) in a UV cell was titrated by the addition of 5-μL increments of a 1.80 mM aqueous solution of Dawson HPT **2b** ($K^+$ salt, pH 5.9). The turbidity of the mixture was monitored by observing changes in absorbance at 775 nm vs. microliters added (Figure 1a). The mixture became turbid after the first addition. After a total of 100 μL (1.80 × 100 × 7 = 1.3 μequiv of negative charge) was added, the resulting precipitate was collected by centrifugation and dried, giving 2.01 mg (100%).

A second titration was performed as described above using as the titrant 100 μL of a 1.80 μM solution of Dawson HTP **2b**, which was also 19 mM in ligand **23a** (i.e., 100 μL contained 5.0 mg of **23a**, 7.7 μequiv of positive charge). There was essentially no turbidity during this latter titration (see Figure 1a).

**Concanavalin A.** Three milliliters (13.0 × 3 × 19 = 0.74 μequiv of positive charge, ignoring the histidine residues) of a stirred 13.0 μM aqueous solution of concanavalin A (0.350 mg/mL, pH adjusted to 7.1 by addition of 1 N NaOH) in a UV cell was titrated by the addition of 5-μL increments of a 1.80 mM aqueous solution of Dawson HPT **2b** ($K^+$ salt, pH 5.9) as described above. After a total of 60 μL (1.80 × 60 × 7 = 0.76 μequiv of negative charge) was added, the resulting precipitate was collected and dried, giving 1.60 mg (100%).

A second titration was performed using as the titrant the Dawson solution which was also 32 mM in ligand **23a** (i.e., 60 μL contained 3.0 mg of **23a**, 7.6 μequiv of positive charge). There was essentially no turbidity during this latter titration (see Figure 1b).

**Poly-L-Lysine.** Poly-L-lysine hydrobromide (Sigma, MW ≃ 17,000, 1.08 mg, 5.2 μequiv of positive charge) was dissolved in 4.00 mL of water. The pH was adjusted to 7.1 by the addition of 1 N NaOH (0.1 mL), and then a 1.00-mL aliquot (1.3 μequiv of positive charge) was removed and diluted with 3.00 mL of water. This was placed in a UV cell and stirred while it was titrated by the addition of 5-μL increments of a 1.80 mM aqueous solution of Dawson HPT **2b** ($K^+$ salt, pH 5.9). After a total of 100 μL (1.3 μequiv of negative charge) was added, the resulting precipitate was collected and dried, giving 1.06 mg (100%).

A second titration was performed as described above using as the titrant 100 μL of a 1.80 mM solution of **2b**, which was also 66 mM in ligand **23a** (i.e., 100 μL contained 10.4 mg of **27a**, 26 μequiv of positive charge). A slight increase in turbidity was observed during this latter titration (Figure 1c).

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**Supplementary Material Available:** Preparation of $[H_3N(CH_2CH_2NH_2)_2CH_2CH_2NH_3]CpTiPW_{11}O_{39}$, $[H_3N(CH_2CH_2NH_2)_3CH_2CH_2NH_3]_4[CpTiPW_{11}O_{39}]_5$, $[H_3N(CH_2)_6NH_3]_2CpTiPW_{11}O_{39}$, $[Me_3N(CH_2CH_2NMe_2)_2CH_2CH_2NMe_3]CpTiPW_{11}O_{39}$, $[Me_3N(CH_2CH_2NMe_2)_3CH_2CH_2NMe_3]_4[CpTiPW_{11}O_{39}]_5$, $[Et_3N(CH_2CH_2O)_2CH_2CH_2NEt_3]CpTiPW_{11}O_{39}$, $[Et_3N(CH_2CH_2O)_2CH_2CH_2NEt_3]_7(CpTiP_2W_{17}O_{61})_2$, $[(CH_3CH(OH)CH_2)_2NHCH_2CH_2NH(CH_2CH(OH)CH_3)_2]_2CpTiPW_{11}O_{39}$, $[(HOCH_2CH_2)_3N(CH_2CH_2O)_2CH_2CH_2N(CH_2CH_2OH)_3]_2CpTiPW_{11}O_{39}$, and $[(HOCH_2CH_2)_3N(CH_2CH_2O)_2CH_2CH_2N(CH_2CH_2OH)_3]_7[CpTiP_2W_{17}O_{61}]_2$ (3 pages). Ordering information is given on any current masthead page.

# Silicon Hydrides and Molybdenum(0) Catalyst: A Novel Approach for Conjugate Reduction of α,β-Unsaturated Carbonyl Compounds

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A novel reducing system comprised of phenylsilane and catalytic amounts of $Mo(CO)_6$ in refluxing THF efficiently effects conjugate reduction of Michael acceptors, including α,β-unsaturated ketones, carboxylic acids, carboxylic esters, amides, and nitriles. The process involves molybdenum-catalyzed hydrosilation, followed by hydrolysis of the intermediate silyl enol ether. Hydride is regioselectively transferred from the hydridosilane to the β-carbon of the substrate, and a proton from water is incorporated into the α-carbon.

The design of composite reducing systems comprised of a relatively nonreactive source of hydride entities, such as group 14 metal hydrides and transition-metal catalysts of groups 8–10, represents a highly useful reduction strategy. This general approach, which is based on the specific delivery of hydride ions from a nonreactive donor to the target functionality, has provided new opportunities for reductive cleavage of allylic heterosubstituents[1] and conjugate reduction of Michael acceptors[2] with superior se-

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