Registry No. **2** (C, D = p-C_6H_5; Ar = p-C_6D_4Cl, X = I), 97170-30-4; **2** (C, D = p-C_6H_5; Ar = p-C_6D_4Cl, X = Br), 97170-31-5; **2** (C, D = p-C_6H_5; Ar = p-C_6D_4Cl, X = Cl), 97170-32-6; **2** (C, D = p-C_6H_5; Ar = p-C_6D_4Cl, X = N_3), 97170-33-7; **2** (C, D = p-$C_6H_4CH_3$; Ar = p-C_6H_4Cl, X = I), 97170-34-8; **2** (C, D = p-C_6D_5; Ar = p-C_6H_4Cl, X = I), 97170-35-9; **2** (C, D = p-C_6H_5; Ar = p-C_6H_4Cl, X = I), 97170-36-0; **3**, 97149-92-3; **4** (isomer 1), 97149-93-4; **4** (isomer 2), 97149-94-5; **4** (isomer 3), 97170-37-1; **4** (isomer 4), 97170-38-2.

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Synthesis, Oxygen-Binding Behavior, and Mössbauer Spectroscopy of Covalently Bound Polyphosphazene–Heme Complexes[1]

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The water-soluble poly(aminophosphazene) $[NP(NHCH_3)_x(NCH_3CH_2CH_2CH_2NH_2)_y]_n$ has been investigated as a polymeric carrier molecule for the covalent attachment of (a) a modified picket fence hemin (**3**) and (b) protohemin chloride 3-(1-imidazolyl)propylamide (**4**). Reduction of the polymer-bound species with dithionite in DMF/H_2O 9:1 or H_2O/ethylene glycol 1:1 yielded the corresponding heme complexes. The oxygenation behavior of these species was examined at −30 °C and at room temperature by electronic absorption spectroscopy. Polymer-bound **4** gave a stable O_2 adduct at −30 °C in DMF/H_2O but was oxidized in ethylene glycol/H_2O. Polymer-bound **3** gave a stable O_2 adduct in DMF/H_2O or ethylene glycol/H_2O only in the presence of excess axial base. Solid films of the two polymer-bound hemes were inert to oxygen for periods of weeks but did react with carbon monoxide to yield carbonmonoxy derivatives. The oxygenation behavior of the cross-linked films swollen with solvent showed a closer resemblance to that of the solution-state systems than to that of the solid state. Dc polarography was performed on the two polymeric hemins, and cathodic shifts relative to the free hemins of >100 mV were seen for both species. Iron-57-labeled derivatives of hemin **3** were prepared, and the oxygenation behavior of the polymer-bound iron-57-labeled species was examined by Mössbauer spectroscopy. The small molecule species $N_3P_3(OPh)_5[N(CH_3)CH_2CH_2CH_2NH_2]$ was synthesized as a model for the high polymer, and the corresponding derivatives with **3** and **4** were prepared.

Polymer-bound metallo compounds are of interest for uses in catalysis,[2] membrane technology,[3] electrode modification,[4] and bioinorganic model chemistry.[5] In this present work, we have explored the use of an inorganic–organic polymer system, based on a polyphosphazene backbone, as a carrier for metalloporphyrins. In an earlier study[6] we examined the influence of a water-soluble aminophosphazene polymer as a *coordination* carrier for heme or hemin.[6] Here, we consider the effect of *covalent* bonding between the water-soluble carrier macromolecule and two metalloporphyrins on the oxygen-binding and electrolytic characteristics of the systems. We were particularly interested in comparisons between the oxidation or oxygenation behavior of these species in the solid state and in solution.

The field of polymer-bound or silica-immobilized metalloporphyrin chemistry is an active and, in some respects, controversial research area. Its development can be traced to the well-known anomaly that hemoglobin or myoglobin bind dioxygen reversibly but simple ferrous compounds are irreversibly oxidized in the presence of oxygen and water. Free heme molecules are also sensitive to Fe(II) → Fe(III) oxidation. Hence, the proteinoid macromolecular "carrier" in hemoglobin or myoglobin is assumed to retard oxidation and permit reversible oxygenation. Various specific roles have been ascribed to the protein molecules, including a physical separation of the heme units to prevent μ-oxo dimer formation, the generation of a hydrophobic pocket around the metalloporphyrin to retard oxidative electron transfer from iron to oxygen, coordination of heme to only one imidazole (histidine) unit to leave the sixth coordination site essentially free for oxygen binding, and the formation of an environment of low acidity, which retards oxidation.

Thus, various model systems have been devised to test each of these influences.[8] The hydrophobic environment and physical separation hypotheses have been tested by Collman[9] and Baldwin[10] with their respective "picket fence" and capped porphyrin species. The requirement for mono(base) coordination was examined by Traylor[11] through the synthesis and flash photolysis of modified hemes with pendent, covalently attached imidazole. The ideas of a physical separation of metalloporphyrin units and the need for a hydrophobic environment were also tested by Wang[12] who embedded a heme ester in a solid, hydrophobic polystyrene matrix and detected reversible oxygen binding. Collman[14] has investigated

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the use of solid imidazolyl–polystyrene films for this purpose. Basolo[13] studied the chemistry of heme molecules that were physically separated on a silica gel surface.

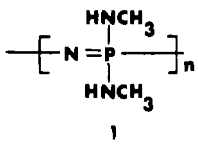
In principle, the attributes ascribed to the carrier protein molecules could be designed into soluble synthetic polymer molecules. (This viewpoint neglects the possibility strongly advocated by many biochemists and molecular biologists that the detailed structure and conformation of globin is the real key to its protective influence.) Thus, Bayer and Holzbach[15] reported that an imidazolyl–heme analogue, covalently bound to poly(ethylene oxide) or to a poly(ethylene oxide)–urethane copolymer, absorbed and released dioxygen readily at 25 °C in dilute aqueous media. However, their initial communication has not yet been supplemented by a more detailed report.

Along the same lines, Tsuchida and co-workers have linked modified hemes,[16] picket fence porphyrins,[17] and Traylor-type[18] complexes to water-soluble synthetic organic polymers and have reported reversible oxygenation in aqueous media at −30 °C. The incorporation of modified heme complexes into micellar bilayers appears to stabilize the Fe(II) state against oxidation, as demonstrated initially by Vasilenko[19] and co-workers and more recently by Tsuchida.[20]

Our approach has been to use a basic, water-soluble carrier polymer linked to a Traylor type pendent imidazolyl–heme derivative and to a variant of Collman's picket fence heme. We have examined oxygenation or oxidation in solution or the solid state by means of UV–visible spectroscopy and Mössbauer spectroscopy. Mössbauer data are vital to the interpretation of mechanisms and structures in systems of this kind because UV–visible data alone can lead to erroneous conclusions. With a view to the possible use of polymer-bound porphyrins in the mediation of electrode processes, we have also examined briefly the electroreduction behavior of these macromolecular systems.

Results and Discussion

Coordinatively Bound Heme Systems. In a previous paper on this topic,[6] we described the influence of carrier polymers **1** and **2** on the response to oxygen of iron(II) protoporphyrin IX chloride in aqueous media. We concluded that polymer **1** functions mainly by providing an alkaline medium for the generation of hematin, hemin hydroxide, or diaquoheme. It also restricts the free diffusion of the metalloporphyrins by forming salt linkages between the side group amine sites and the free carboxylic acid groups of hemin chloride. Polymer **1** had little, if any, retarding effect on the oxidation of heme to hemin in aqueous media at 25 °C.



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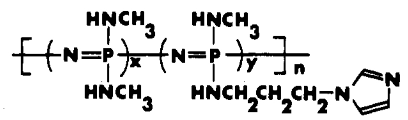
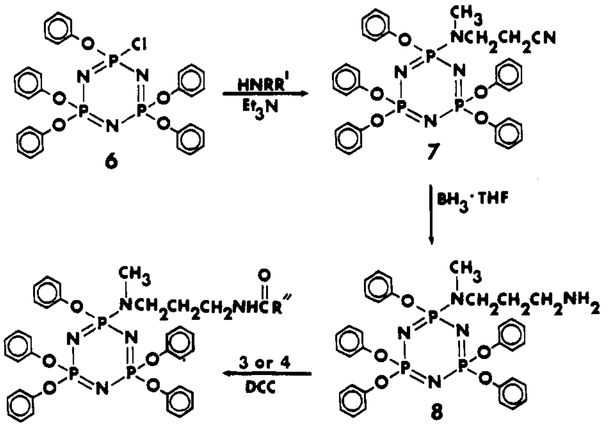


2

x : 10y ; n ≈ 15000

Polymer **2** formed a strong coordinative bond to the iron centers of heme or hemin through the pendent imidazolyl units and also through salt linkages that involved the carboxylic acid groups. In aqueous media, polymer **2** had no discernible retarding effect on the oxidation of heme to hemin at 25 °C. However, films of polymer **2**–heme complexes showed electronic spectral evidence for reversible oxygenation at 25 °C. Moreover, the same films underwent reversible carbon monoxide complexation. It was subsequently shown from Mössbauer data that a key facet of this apparent cycling is the ability of polymer **1** or **2** to reduce hemin chloride to heme in the solid state in vacuo. Thus, the supposed reversible oxygenation probably involves oxidation of Fe(II) to Fe(III) followed by reduction of Fe(III) *by the carrier polymer* back to Fe(II) in the absence of oxygen. This illustrates why Mössbauer data are essential for an understanding of phenomena of this type.

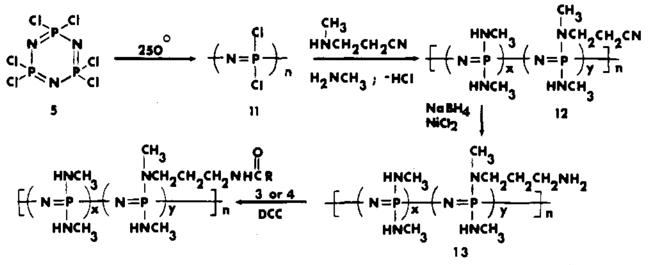
Scheme I



9 Where $R'' = CH_2\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}\overset{H}{\overset{|}{N}}\text{-}C_6H_4\text{-}$Porphyrin (From 3)

10 Where $R'' = CH_2\text{-}CH_2\text{-}$Porphyrin (From 4)

Scheme II



x : 10y

n : 15000

14 Where $R = CH_2\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}\overset{H}{\overset{|}{N}}\text{-}C_6H_4\text{-}$Porphyrin (From 3)

15 Where $R = CH_2\text{-}CH_2\text{-}$Porphyrin (From 4)

Synthesis of Covalently Bound Polymer–Metalloporphyrin Systems (General Principles). The coordinative linkage of a metalloporphyrin to a polymer such as **2** through a metal–imidazole linkage is unsatisfactory from several points of view. First, it is not possible in a solution system to ensure that only one imidazole unit binds to a metalloporphyrin center. The possibility is quite high that two imidazole residues, from the same or different polymer chains, could bind to form a hexacoordinate complex. This would be in conflict with the known requirements for reversible oxygenation. Polymer **2** contained one imidazolyl pendent group for every eleven repeating units along a unit chain. Second, polymer **2** contains no structural features that would provide a hydrophobic environment for the metalloporphyrin. And third, although polymer coiling in the solid state might provide some steric protection to retard μ-oxo dimer formation, it is unlikely that dimerization would be prevented in solution.

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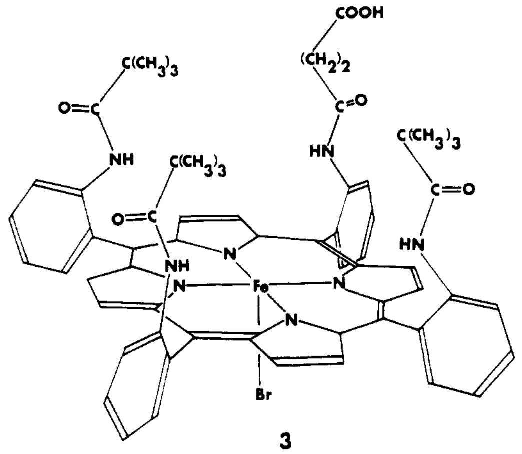
In the present work, we have attempted to overcome some of these problems by the covalent linkage of metalloporphyrins **3** and **4** to a polyphosphazene bearing a reactive pendent alkylamino group (**13**). Metalloporphyrin **4** is a semisynthetic species derived from protohemin chloride. It was first described by Traylor.[11c] Compound **3** is a totally synthetic metalloporphyrin first reported by Tsuchida[17] and co-workers, based on Collman and Gagne's earlier picket fence porphyrin. It was anticipated that the aliphatic carboxylic acid group in both compounds would couple readily to polymer **13**. As in most new poly(organophosphazene) synthetic work,[21] it was necessary to first develop the required synthetic procedures at the small molecule model level (Scheme I) before attempting the coupling reaction with the chosen macromolecular substrate (Scheme II).



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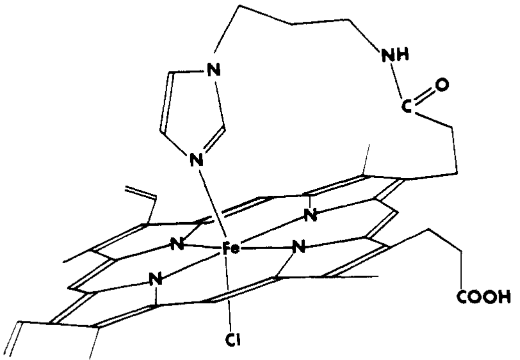

4

Coupling Reaction at the Small Molecule Level. It was vital to establish initially if coupling reactions between aminophosphazenes and carboxyl-containing hemins could be carried out. Earlier work in our laboratory[22] had shown that the condensation coupling between a simple carboxylic acid and an aminophosphazene did not take place readily unless the amino residue was separated from the phosphazene skeleton by methylene (or aryl) spacer groups. Thus, substrate **7** (Scheme I) was prepared from **5** by treatment with sodium phenoxide, followed by reaction with 3-(methylamino)propionitrile and reduction of the cyano group. The five phenoxy blocking groups were incorporated into **6**, **7**, and **8** because species **6** , with only one active site per ring, can be prepared readily.[23] It was considered essential to synthesize a model with only one active side unit in order to simplify the structural characterization. Species **8** was then coupled through the carboxylic acid groups with the two metalloporphyrins **3** and **4** with the use of *N*,*N*'-dicyclohexylcarbodiimide (DCC),[27] ethyl chloroformate, or pivaloyl chloride, as described in the Experimental Section, to yield species **9** and **10**.

Species **7**–**10** were characterized by a combination of mass spectrometry, infrared spectroscopy, UV spectroscopy and, ^{1}H and ^{31}P NMR spectroscopy. The data are summarized in the Experimental Section.

Compound **7** showed a strong CN stretch at 2235 cm^{-1} in the infrared spectrum, which disappeared following reduction to compound **8**. Compound **8** yielded a strong NH stretch at 3200 cm^{-1}. The coupled products **9** and **10**, where units R are the residues derived from **3** and **4**, showed strong stretches indicative of amides at 1650–1660 cm^{-1}. All the compounds showed the characteristic PN stretch at between 1100 and 1300 cm^{-1}.

The ^{1}H NMR spectrum of compound **7** was consistent with the assigned structure and was characterized by the splitting of the doublet assigned to the *N*-methyl protons at 2.55 ppm into a doublet by phosphorus (J_{PNCH} = 11 Hz). After **7** was reduced to **8**, two extra sets of resonances at 3.76 and 2.61 ppm, assigned to the aminomethylene group, were detected. Those at 3.76 ppm underwent proton exchange following the addition of D_2O. The coupled species **9** and **10** were paramagnetic, and satisfactory ^{1}H NMR spectra were not obtained.

The ^{31}P NMR spectrum of **7** consisted of an AB_2 spectrum centered at 18.5 and 9.3 ppm (J_{AB} = 75 Hz). Reduction to **8** yielded another AB_2 pattern at 18.71 and 9.28 ppm (J_{AB} = 72.5 Hz). Such small changes are consistent with the new species **8** having almost the same environment about phosphorus as **7**.

The UV spectra of the coupled species **9** and **10** were identical with those of **3** and **4** in the region 400–700 nm, which indicated that the metalloporphyrins underwent no destructive reactions under the coupling conditions employed (compound **9**, 419, 508, 577, 648, 675 nm in $CHCl_3$ solution; compound **10**, 408, 540, and 563 nm in DMF/H_2O 70:30). The mass spectra of all the species showed strong parent ions, and all compounds gave satisfactory elemental analyses (see Experimental section).

Synthesis and Coupling Reactions of the High Polymers. Polymer **12** (Scheme II) was prepared by the addition of a controlled amount of 3-(methylamino)propionitrile to a dilute solution of poly(dichlorophosphazene), followed by the addition of the resultant polymer to a large excess of methylamine[26] in THF. The product polymer was a colorless, alcohol- and water-soluble species. Its structure was deduced by high-field ^{1}H NMR analysis, which provided evidence (see Experimental Section) that little or no displacement of 3-(methylamino)propionitrile side groups had taken place. The two types of side group were present in a ratio of 19:1, with the methylamino groups predominating.

Reduction of the pendent cyano group proved to be more difficult for polymer **12** than for model compound **7**. Treatment with $BH_3 \cdot THF$[22,24] did not yield the expected product. Neither did an attempted reduction with lithium aluminum hydride or catalytic hydrogenation over Ni/C or Pd/C. The reduction was eventually carried out successfully by the technique of Suzuki and co-workers,[25] which involves reaction of the polymer in methanol with nickel chloride and sodium borohydride, followed by treatment with hydrochloric acid. ^{31}P NMR spectra of polymers **12** and **13** show broad singlets in a ratio consistent with the structures indicated in Scheme II.

The carboxylic acid-bearing hemins **3** and **4** were coupled to polymer **13** in ethanol solution at pH 4.5 with the use of *N*,*N*'-dicyclohexylcarbodiimide (DCC)[27] or 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride. The coupling reactions were also effected in DMF with the use of DCC or ethyl chloroformate as the activating agent. The polymer-bound metalloporphyrins **14** and **15** were then freed from unreacted **3** or **4** by dialysis against aqueous ethanol. The degree of coupling of metalloporphyrin to the polymer was deduced from control plots of the absorbances in the Soret region of the UV spectrum for the free metalloporphyrins as a function of concentration. Ap-

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Table I. UV Data for Polymeric Hemes **14** and **15**

heme derivative	solvent	temp, °C	λ_{max}, nm		
			deoxy	oxy	carbonmonoxy
15	DMF/H_2O (9:1)	−30	526, 556	540, 574	536, 566
15	DMF/H_2O (9:1)	25	556 (br)	oxidized	536, 566
15[a]	film	25	528, 558	538, 570	534, 564
15	ethylene glycol/H_2O (1:1)	−30	526, 556	oxidized	538, 566
14[b]	ethylene glycol/H_2O (1:1)	−30	534, 562	536	540
14[b]	DMF/H_2O (9:1)	25	532, 564	538	542
14[c]	film	25	530		542

[a] All films were dried under high vacuum for 12 h before use. [b] With added 1-methylimidazole (4 equiv). [c] With or without added 1-methylimidazole.

proximately 1 hemin residue was linked to the chain for every 200 repeating units.

Oxygenation–Oxidation Studies for Solutions of 15. Electronic Spectra. The data are summarized in Table I. Solutions of **15** (10^{-4} to 10^{-5} M in metalloporphyrin) were reduced to the Fe(II) forms by treatment with dithionite in quartz spectroscopy cells. Compound **15** yielded a 5-coordinate (Fe(II)) species (broad absorption centered at 556 nm in 9:1 DMF/H_2O) after dithionite reduction,[18] which changed to a 6-coordinate species (absorption 526 and 556 nm) after being cooled to −30 °C.[28] Addition of O_2 to the cooled solution brought about a dramatic change in the spectrum, with the appearance of new absorbances at 540 and 574 nm. These changes could be reversed by bubbling nitrogen through the solution, and the process was repeatable for at least three cycles. Furthermore, addition of CO to the oxygenated species yielded the distinctive spectrum of a carbonmonoxyheme (absorbances at 536 and 566 nm).[18] The carbonmonoxyheme spectrum was also obtained when CO was introduced into a solution of the cooled non-oxygenated, reduced form of **15**. These results are compatible with the conclusion that the reduced form of **15** at −30 °C in 9:1 DMF/H_2O undergoes reversible oxygenation rather than oxidation.

The oxygenated complex obtained from **15** in 1:1 DMF/H_2O was stable for at least 40 min at −30 °C, although it underwent rapid, irreversible oxidation at temperatures above −15 °C. At 25 °C the lifetime of the oxygenated complex was only 2–3 min. This behavior is similar to that reported by Tsuchida and coworkers for a species similar to **4** but containing an extra two methylene groups in the chelating arm, covalently linked to a poly(ethylene glycol) glycine carrier polymer.[18a]

A criticism often leveled at results of this type is that the apparent reversible oxygenation is in reality traceable to re-reduction of an oxidized (Fe(III)) form by excess dithionite present in the system. Thus, for experiments of this type to be meaningful, it is essential that (a) the addition of dithionite should be carried out slowly and carefully to avoid the presence of excess reagent (generally a 5:1 ratio of dithionite to hemin is appropriate) and (b) the absorbance at 350 nm should be monitored for evidence of excess dithionite. These precautions were taken in the present work, with the use of freshly prepared solutions of dithionite for each set of measurements.

A change in the solvent system to 1:1 ethylene glycol/water resulted in a rapid *oxidation* of reduced **15** in the presence of oxygen at temperatures as low as −50 °C.

Oxygenation–Oxidation Studies for Solutions of 14. Electronic and Mössbauer Spectra. Dithionite reduction of compound **14** in 9:1 DMF/H_2O at 25 °C yielded a spectrum (absorption at 565 and 535 nm) similar to that of the 4-coordinate Fe(II) species reported by Collman for $FeT_{piv}PP$ in benzene.[9] However, in DMF/H_2O high-spin 5- or 6-coordination systems that contain solvent molecules are also possible, and these would yield similar spectra. Addition of oxygen to reduced **14** resulted in instant oxidation to the Fe(III) form at temperatures from −30 °C to +25 °C. This result can be interpreted to mean that the methylamino side groups and the backbone nitrogen atoms show little or no tendency to coordinate to the fifth and sixth coordination sites of the picket fence heme. This is surprising in view of the known tendency of $[NP(NHCH_3)_2]_n$ to coordinate strongly to transition metals in aqueous media.[6,29]

Table II. Mössbauer Data[a]

species	center shift δ, mm/s	quadrupole splitting ΔE_Q, mm/s
deoxy-**14**	0.40	1.09
oxy-**14**[b]	0.28, 0.42	2.12, 1.05
carboxy-**14**	0.30	0.31
HbO_2	0.24	2.24
Hb	0.91[c]	2.26

[a] All spectra were obtained at 77 K in frozen solutions of DMF/H_2O. Values are relative to metallic iron. A useful spectrum was usually obtained in less than 3 h. [b] Mixture of deoxy and oxy forms in ≃ 3:2 ratio. [c] At 4.2 K.

However, reduction of **14** in the presence of added 1-methylimidazole (4 equiv/Fe unit) at 25 °C yielded a spectrum indicative of the 6-coordinate complex, which contained 2 molecules of the axial base (absorption at 534 and 562 nm).[9] Addition of oxygen to this solution at 0 °C caused a shift of the intense band at 534 nm to 538 nm and a disappearance of the less intense band at 562 nm. This is compatible with the formation of an oxygenated derivative of reduced **14**.[30]

The addition of CO to a solution of either the deoxy or oxy forms of reduced **14** led immediately to the formation of a carbonmonoxyheme spectrum (absorbance at 542 nm). This change was accompanied by the appearance of a bright cherry red color. The spectrum of the carbon monoxide adduct could not be changed by bubbling either oxygen or nitrogen through the solution.

Cycling between the deoxy and oxy forms of reduced **14** was detected for at least three cycles at 25 °C following successive treatments with oxygen and nitrogen, although some decomposition occurred during each cycle. The oxygenated derivative of reduced **14** was stable at 25 °C for ≃2 h. This is a somewhat lower stability than was reported by Tsuchida[17] for oxygenated **3** linked to a ternary block copolymer in which heme units are completely immobilized in a micellar core. Thus, the lower stability of our system may be a consequence of the inability of the carrier polymer to coordinate to the fifth and sixth coordination sites of the Fe(II) or may reflect the lack of hydrophobicity around the active site.

A change in the solvent system for **14** from 9:1 DMF/H_2O to 1:1 ethylene glycol/H_2O decreased the stability of the oxyheme form and caused irreversible oxidation within 5 min. Increases in the concentration of axial base did not increase the stability.

For the reasons discussed earlier, it was considered prudent to examine the Mössbauer spectra of the species prepared in this

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(30) Tsuchida[17] has reported that the oxygen affinity of $FeT_{piv}PP$ is very sensitive to the axial ligand to Fe ratio if the axial ligand is 1-ethylimidazole and the deoxygenated species is 6-coordinate. Collman[9a] has indicated that the Q-band shift of oxygenated $FeT_{piv}PP$ compared to that of the deoxygenated form is 11 nm. The parameter DO (degree of oxygenation) was, therefore, defined by Tsuchida as DO = [Q-band shift × 100%]/11. A similar result may be obtained by comparisons of the Soret band shifts. In the present work the application of these principles suggests a value of ≃36% oxygenation, assuming a Q-band shift of 4 nm. This value is consistent with the Mössbauer data.

work. The natural abundance of iron-57 in these systems was found to be too low for meaningful comparisons to be made. Hence, the metalloporphyrin units in compound **14** were modified by the introduction of iron-57 (see Experimental Section). The Mössbauer data obtained for polymer **14** and its derivatives, together with some comparisons, are given in Table II.

The most important comparison is between the spectra of oxygenated reduced **14** and oxyhemoglobin. The results are compatible with a species in which both oxygenated reduced **14** and the non-oxygenated bis(1-methylimidazole) derivative are both present, with about 36% of the mixture consisting of the oxygenated form.[30] The Mössbauer data for the carbonmonoxy and deoxy forms of reduced **14** are in good agreement with the literature values for $FeT_{piv}PP$, both with respect to isomer shift and quadrupole splitting.[9a] The closeness of the agreement allows us to place some confidence in our assignments derived from the electronic spectral data.

Solid-State Behavior of 14 and 15. In contrast to the results described above, marked differences were found for the oxygenation reactions of **14** and **15** in the solid state. Specifically, removal of solvent from solutions of reduced **14** or **15** yielded films that gave UV spectra consistent with the presence of 6-coordinate species (**14**, 530 nm; **15**, 528, 558 nm). These spectra remained unchanged following exposure of the film to oxygen for periods of weeks. Exposure to carbon monoxide brought about a complete conversion to the carbonmonoxy derivatives within 3 h (**14**, 542 nm; **15**, 536, 566 nm). This assignment was confirmed in the case of **14** by the appearance of a CO stretching peak at 1960 cm^{-1} in the infrared spectrum.[9a] Heating of carbonmonoxy-**14** in vacuo gave a 5-coordinate species that, following exposure to oxygen, yielded a spectrum consistent with a mixture of oxy-**14** and the 6-coordinate species described above (broad absorbances at 534 and 565 nm). Removal of the gas from the cell, followed by admission of carbon monoxide, caused the reappearance of the carbonmonoxy-**14** spectrum.

An attempt was made to explain the differences between the solution- and solid-state behavior of these polymers by an examination of the permeability of the films to oxygen. An attempt was also made to identify the origin of the sixth ligand in the 6-coordinate species. We conclude that the inertness of the polymeric hemes to oxygenation or oxidation in the solid state is not due to the impermeability of the polymeric matrices to oxygen. Suspensions were made of the species $Fe_4S_4(SC_4(SC_4H_9\text{-}t)_4{}^{2-}$ in polymer **13**. The iron sulfur anion has a characteristic absorbance at 418 nm and is extremely sensitive to oxygen.[31] Exposure of this film to oxygen brought about a decomposition of the iron sulfur species in minutes. Thus, while the oxidation or oxygenation of **14** or **15** may be slowed in the solid state, a reduced transmission of oxygen through the films cannot explain the low reactivity of **14** and **15** toward oxygen.

The identity of the sixth ligand in the 6-coordinated films of **14** and **15** is an important question. This ligand is believed to be a basic nitrogen atom from the polymer backbone or from a side-chain methylamino residue. Evidence for this supposition included the following: (a) Identical results are obtained when a variety of solvents are used to prepare the films (ethanol, ethanol/water mixtures and THF). This suggests that the sixth coordinating ligand is not derived from the solvent. (b) Films prepared from reduced samples of **3** or **4** in polystyrene ($M_n = 10^6$) underwent rapid *oxidation* when exposed to oxygen. (c) Films prepared from reduced samples of **3** or **4** in polymer **1** or **13** yielded 6-coordinate species. It seemed likely that the sixth ligand was only weakly bound, and this possibility was examined by the following experiments. Samples of reduced **14** or **15** were lightly crosslinked by γ irradiation (see Experimental Section) and were swollen with degassed DMF/H_2O. The UV spectra of these samples gave spectra that were consistent with a 5-coordinate species for **15** (556 nm) and a possible 4-coordinate modification for **14** (564, 535 nm). These spectra are compatible with the belief that the sixth ligand (and, in the case of **14**, in the absence of excess added axial base, the fifth ligand also) is derived from the polymeric carrier and is only weakly bound. These results are consistent with other reports on the mobility of polymeric ligands in swollen gels.[9b]

Dc Polarography. Polymeric metalloporphyrins are of interest as electrocatalysts. The half-wave reduction potentials of the Fe^{3+}/Fe^{2+} couple of the two polymeric hemins were, therefore, determined in DMF by dc polarography. Both polymeric hemins show a distinct cathodic shift compared to the free hemins (**15** from -0.34 to -0,46 V and **14** from -0.10 to -0.19 V with respect to SCE). These differences were maintained at scan rates of 0.05 V/min up to 0.5 V/min. The differences cannot be ascribed to changes in the viscosity of the solutions. Solutions of mixtures of **3** and **4** and the carrier polymer **13** give a Fe^{3+}/Fe^{2+} reduction wave at the same potentials as the free hemins. The carrier polymer **13** itself is electroinactive in the potential range examined (0 to -2.5 V). Mixtures of free hemin **4** and polymeric hemin **15**, or of free hemin **3** and polymeric hemin **14**, show two distinct reduction peaks at the potentials listed above.

Solutions of the model compounds **10** and **9** gave reduction waves at -0.32 and -0.11 V, respectively. Therefore, we conclude that the larger cathodic shifts found for the polymeric derivatives are not due to electronic effects. Instead, the differences are probably due to the special environment generated by the coiling of the macromolecular chains in solution. In turn, this leads to a slight resistance to reduction. These results may be significant for the preparation of polymer-coated electrodes. Studies are in progress to examine this possibility with other polyphosphazene polymers.

Experimental Section

Reagents. Hexachlorocyclotriphosphazene (**5**) (mp 110-112 °C) was obtained from a trimer-tetramer mixture (Ethyl Corp.) after two vacuum sublimations at 60 °C (0.5 torr), two recrystallizations from heptane, and two additional vacuum sublimations. Poly(dichlorophosphazene) (**11**) was prepared by the thermal polymerization of $(NPCl_2)_3$ at 250 °C for an 8-24-h period in a sealed Pyrex tube (20 × 2.5 cm). Typically, less than 25% conversion to the high polymer was attempted, and the unreacted trimer was then recovered by sublimation at 60 °C (0.5 torr) for 12-24 h. The polymer was soluble in organic media such as toluene or tetrahydrofuran.

Methanol was distilled from $Mg(OCH_3)_2$ before use. Tetrahydrofuran was boiled at reflux over, and distilled from, sodium/benzophenone. Triethylamine was dried with, and distilled from, CaH_2 and BaO before use. Pyridine was distilled from calcium hydride. Dimethylformamide (DMF) was distilled under reduced pressure, and then stored over 4-Å molecular sieves. Deionized water was used for all aqueous solutions. Sodium dithionite (J. T. Baker) was used as received and stored in a glovebox. Fresh solutions of this reagent in degassed deionized water were prepared daily. *N*-(3-Bromopropyl)phthalimide (Aldrich), protohemin chloride (Sigma), and hydrazine (Mallinkrodt) were used as received. Methylamine (Matheson) was condensed over and distilled from sodium potassium alloy before use. Phenol (Aldrich) was sublimed once.

3-(1-Imidazolyl)propylamine dihydrochloride was prepared by the method of Schwan.[32] $^{57}Fe(II)$ chloride was prepared by the method of Winter[33] from iron-57 powder (New England Nuclear). Compound **3** was prepared by the method of Tsuchida[17] starting from *meso*-tetrakis-(*o*-aminophenyl)porphyrin.[9] Gram quantities of the desired tetra-α isomer could be separated by the method of Lindsey.[34] Compound **4** was prepared by a modification of the method of Traylor et al.[11c]

Electronic absorption spectra were recorded on a Hewlett-Packard 8450A instrument with the use of glass 1-cm cells equipped with Teflon stopcocks. All procedures that involved the use of Fe(II) species were carried out with the use of degassed solvents under an atmosphere of purified nitrogen. Mössbauer spectra were recorded at 77 K for frozen solutions or for dehydrated solids freeze-dried from H_2O and with samples placed in 7-mm polyethylene containers. Infrared spectra were obtained by means of a Perkin-Elmer spectrometer with samples examined as KBr disks or as thin films on NaCl plates. Proton-decoupled ^{31}P NMR spectra were obtained at 40 MHz with a JEOL PS 100FT spectrometer equipped with a Nicolet 1080 data processing system. The

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solvent was THF, and chemical shift positions are reported relative to 85% H_3PO_4, where positive chemical shifts represent deshielding. A D_2O capillary lock was used. Proton NMR spectra were recorded at 60 MHz on a Varian EM-360 or at 200 MHz on a Bruker WP-200 spectrometer in the Fourier transform mode. Mass spectra were obtained by the use of a Finnegan AEI MS 902 mass spectrometer operating at an ionization potential of 30 eV. Elemental analyses were obtained by Galbraith Laboratories, Knoxville, TN. Approximate polymer molecular weights were estimated by use of a Waters Associates ALC-201 gel permeation chromatography instrument fitted with a 122 × 1 cm 10^5 Å Styragel column for use with THF at a flow rate of 2.4 mL/min. Approximate calibration of the columns was accomplished by means of narrow molecular weight distribution polystyrene standards obtained from Waters Associates.

Synthesis of $N_3P_3(OPh)_5[N(CH_3)CH_2CH_2CN]$ (7). To a solution of $N_3P_3(OPh)_5Cl$[23] (2 g, 3.1×10^{-3} mol) in dry THF (50 mL) was added excess freshly distilled triethylamine. 3-(Methylamino)propionitrile (1.05 g, 4×10^{-3} mol) was added via syringe, and the solution was heated to reflux. The reaction was allowed to proceed until all the $N_3P_3(OPh)_5Cl$ had been consumed (as monitored by ^{31}P NMR). The solution was cooled and filtered, and the solvent was removed under vacuum. The residue was dissolved in dichloromethane, the resulting mixture extracted with water and dried with $MgSO_4$, and the solvent removed. The crude product was chromatographed on silica gel (CH_2Cl_2) with elution by CH_2Cl_2. The product is a colorless oil (yield 45%). Anal. Calcd for $C_{34}H_{32}N_5O_5P_3$: C, 59.73; H, 4.69; N, 10.24. Found: C, 59.58; H, 4.73; N, 10.12. IR: 2235, 1280–1250, 1200–1160 cm^{-1}. ^{31}P NMR: 18.5 (AB_2 pattern P(O)(N)), 9.3 ppm ($P(O)_2$, J_{PP} = 75 Hz). ^1H NMR ($CDCl_3$): 7.5 (m, 25 H), 3.0 (dt, 2 H), 2.56 (d, 3 H, J_{PNCH} = 11 Hz), 2.05 (t, 2 H) ppm. MS: *m/e* calcd 863, found 863.

Synthesis of $N_3P_3(OPh)_5[N(CH_3)CH_2CH_2CH_2NH_2]$ (8). To a solution of **7** (1.0 g, 1.46×10^{-3} mol) in dry THF under a nitrogen atmosphere was added $BH_3 \cdot THF$ (3 mL of a 1.0 M solution). The solution was stirred at room temperature for 24 h, followed by the addition of 5 mL of CH_3OH. (*Caution*!) The solvent was removed under reduced pressure, and the crude product was chromatographed on basic alumina with elution by chloroform/methanol (10:1). The product was isolated as a colorless oil (yield 90%).

Anal. Calcd for $C_{34}H_{36}N_5O_5P_3$: C, 59.38; H, 5.24; N, 10.12. Found: C, 59.13; H, 5.33; N, 10.01. IR: 3300, 1280–1250, 1200–1160 cm^{-1}. ^{31}P NMR: 18.71 (AB_2 pattern P(O)(N)), 9.28 ppm ($P(O)_2$, J_{PP} = 72.5 Hz). ^1H NMR ($CDCl_3$): 7.5 (m, 25 H), 3.76 (b, 2 H), 2.9 (m, 2 H), 2.61 (m, 2 H), 2.58 (d, 3 H), 1.56 (m, 2 H) ppm. MS: *m/e* calcd 867, found 867.

Synthesis of the Phosphazene Picket Fence Hemin Complex (9). $FeT_{piv}SP \cdot Br$ (3) (50 mg, 3.83×10^{-5} mol) was dissolved in 50 mL of dry CH_2Cl_2 (freshly distilled from CaH_2). Triethylamine (7.66×10^{-5} mol, 7.75 mg) was added, followed by 8.4 mg of ethyl chloroformate, all at 0 °C. The solution was stirred for 1 h. A solution of $N_3P_3(OPh)_5[N(CH_3)CH_2CH_2CH_2NH_2]$ (25 mg, 3.64×10^{-5} mol) in dry CH_2Cl_2 was added in one portion, and the solution was stirred for 2 h at room temperature. The solvent was removed at reduced pressure, the residue dissolved in chloroform, and the resulting mixture washed with dilute HCl and dried with $MgSO_4$. The solvent was removed. The crude product was chromatographed on silica with chloroform/methanol (9:1) as eluent. The first band to be removed was the desired product (55% yield). Anal. Calcd for $C_{98}H_{94}N_{13}O_{10}P_3FeBr$: C, 65.25; H, 5.26; N, 10.20. Found: C, 65.50; H, 5.01; N, 10.85. IR: 1650, 1280–1260, 1200–1170 cm^{-1}. UV ($CHCl_3$): 419, 508, 577, 648, 675 nm.

Synthesis of the Phosphazene Complex of 4 (10). Compound **4** (60 mg, 7.89×10^{-5} mol) in dry chloroform (15 mL) under an atmosphere of nitrogen was added to **10** (54 mg, 7.89×10^{-5} mol). The solution was cooled in ice, and a solution of dicyclohexylcarbodiimide (DCC) (16 mg, 15.78×10^{-5} mol) in $CHCl_3$ (2 mL) was added via syringe. The solution was stirred at 0 °C for 2 h, followed by stirring at room temperature for 4 h. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column ($CHCl_3$). The product eluted as a red band with $CHCl_3/CH_3OH$ 95:5 as eluent. Species **10** was isolated as a dark red-purple solid (≃60% yield). Anal. Calcd for $C_{74}H_{75}N_8O_7P_3FeCl$: C, 62.54; H, 5.05. Found: C, 62.63; H, 4.98. IR: 1660, 1270–1250, 1200–1170 cm^{-1}. UV (DMF/H_2O 70:30): 408, 540, 563 nm.

Synthesis of Polymer 12. Poly(dichlorophosphazene) (5.9 g; 0.1 mol of chloride) was dissolved in THF (500 mL) under an atmosphere of dry nitrogen. 3-(Methylamino)propionitrile (0.84 g, 0.01 mol) was added slowly via syringe. Triethylamine (5 mL) was then added, and the solution was stirred at room temperature for 18 h. Methylamine (50 mL) was condensed over sodium spheres and then recondensed into THF (1000 mL) in a 3-L three-necked flash equipped with an overhead stirrer, dry ice condenser, and a nitrogen inlet. The solution was cooled in ice, and the partially substituted polymer was added via an addition funnel. The solution was stirred at 0 °C for 3 h and at room temperature for 24 h. The flask was opened to the atmosphere to allow excess methylamine to escape. The solution was then concentrated, the precipitate was shaken with 400 mL of water, and the resultant cloudy solution dialyzed for 48 h against deionized water. The dialysis tubes were removed, and the polymer solution was concentrated to dryness to yield the desired polymer as a water- and methanol-soluble, clear, film-forming material. Anal. Calcd for $[NP(HNCH_3)_{1.9}(NCH_3CH_2CH_2CN)_{0.1}]_n$: C, 27.84; H, 7.59. Found: C, 27.48; H, 7.47. IR: 3500, 2230, 1220, 1100 cm^{-1}. ^{31}P NMR: 8.04 (s, $P(N)(N^1)$), 6.10 ppm (s, $P(N)_2$) (ratio ≃ 1:9). ^1H NMR (CD_3OD): 3.2 (m, br), 2.55 (s, br), 2.05 (t) ppm. M_n (GPC) ≃ 1.3×10^5.

Synthesis of Polymer 13. To a solution of **12** (1.12 g; 1.93×10^{-3} mol of CN) in 99% methanol (200 mL) was added sodium borohydride (0.29 g, 9.65×10^{-3} mol) and nickel(II) chloride hexahydrate (0.91 g, 3.86×10^{-3} mol). The solution turned black instantly, with vigorous evolution of hydrogen. The mixture was stirred at 25 °C for 1 h, and hydrochloric acid (50 mL, 3 N) was then added. A clear, homogeneous, green solution was obtained within 5 h. Following dilution with 99% methanol (200 mL), the solution was dialyzed against deionized water in cellulose tubing for 48 h with frequent water changes. After 24 h, no further red precipitate was obtained when the dimethyl glyoximate test for Ni(II) was used.[35] Evaporation of the solvent followed by drying under high vacuum yielded the product as a clear film-forming elastomer. Anal. Calcd for $[NP(HNCH_3)_{1.9}(NCH_3CH_2CH_2CH_2NH_2)_{0.1}]_n$: C, 27.61; H, 7.94. Found: C, 27.23; H, 7.87. IR: 3500, 1226, 1110 cm^{-1}. ^{31}P NMR: 8.25 (s, $P(N)(N^1)$), 6.25 ppm (s, $P(N)_2$) (ratio ≃ 1.9). ^1H NMR (CD_3OD): 3.0 (m, br), 2.55 (m, vbr), 1.7 (m, br) ppm. M_n (GPC) ≃ 1.2×10^5.

Synthesis of Polyphosphazene-Bound Hemins 14 and 15. To a solution of polymer **13** (120 mg; 2.06×10^{-4} mol of NH_2) in DMF (10 mL) was added **4** (50 mg, 6.34×10^{-5} mol). The solution was cooled to 0 °C, and a solution of 1-ethyl-3-(3-(dimethylamino)propyl)carbodimide hydrochloride (EDC) (24 mg; 1.25×10^{-4} M in DMF (1 mL)) was added via syringe. The solution was stirred at 0 °C for 2 h, followed by stirring at room temperature for 24 h. The polymer solution was diluted with 100 mL of deionized water and was dialyzed in cellulose tubing against deionized water/methanol with frequent extractant changes during 48 h. Monitoring of the extracellular solvent by UV spectroscopy detected no hemin after the fourth solvent change. The polymer solution was removed from the dialysis tubing, concentrated in vacuo, and precipitated into acetone. The hemin content was estimated from a control plot to be approximately 1 hemin residue per 200 repeating units. Analysis by thin-layer chromatography ($CHCl_3$/MeOH 9:1 on silica) showed the absence of free hemin.

Cross-Linking of Heme Polymers 14 and 15. In a typical experiment, solutions of **14** and **15** in THF were treated with sodium dithionate, and films were cast on glass slides in a drybox. When thoroughly dry, the glass slides were placed in a thick-walled glass ampule and the ampule was evacuated and sealed. The samples were irradiated with 0.6 M rd (dose rate of 4×10^5 rd/h) of ^{60}Co γ radiation at −78 °C. The irradiations were performed at the Breazeale reactor facility at The Pennsylvania State University. The polymer samples were peeled from the glass and placed in quartz cells fitted with septa. The cells were evacuated, and the appropriate degassed solvents were added via syringe.

Electronic Spectroscopy. In a typical experiment, a sample of **14** or **15** was dissolved in the appropriate solvent in a quartz cell fitted with septa or gas-tight Teflon stopcocks. The final concentration of **14** or **15** was between 10^{-4} and 10^{-5} mM in metalloporphyrin. Each sample was degassed by bubbling purified nitrogen through the solution for 5 min. In the case of **14**, excess organic base (4 equiv) was added by microsyringe at this stage. It is important to note that the normal buffering of aqueous hemin solutions to basic pH is not necessary for the poly(aminophosphazene) systems because aqueous solutions of these species are inherently buffered to pH 7–8.

The cells containing Fe(III) solutions were reduced by the addition of a 5 M solution of freshly prepared and degassed $Na_2S_2O_4$ in the appropriate solvent system. The titration with the reductant and formation of the Fe(II) species were monitored through the examination of the 500–700-nm region of the spectrum. The solutions were then cooled, and oxygen or carbon monoxide was introduced via a gas-tight syringe. Removal of oxygen from the oxygenated species was accomplished by bubbling purified nitrogen through the solutions for several minutes.

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Catalytic Reduction of NO_2^- by HCOOH in the Presence of $MoO_2(S_2CNEt_2)_2$ and $MoO(S_2CNEt_2)_2$

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The reduction of NO_2^- to N_2O by HCOOH in *N*,*N*-dimethylformamide (DMF) in the presence of $MoO(S_2CNEt_2)_2$ or $MoO_2(S_2CNEt_2)_2$ as a catalyst has been accomplished for the first time. The reaction of $MoO(S_2CNEt_2)_2$ with NO_2^- in DMF rapidly proceeds to afford $MoO_2(S_2CNEt_2)_2$ and $N_2O_2^{2-}$, the latter of which may be produced by the dimerization of a possible intermediate NO^-. Both $MoO_2(S_2CNEt_2)_2$ and $N_2O_2^{2-}$ further react with HCOOH to give $MoO(S_2CNEt_2)_2$ and N_2O, respectively. The catalytic reaction obeys first-order kinetics with respect to the concentration of $MoO(S_2CNEt_2)_2$ or $MoO_2(S_2CNEt_2)_2$. The rate-determining step is the reduction of $MoO_2(S_2CNEt_2)_2$ by HCOOH, giving $MoO(S_2CNEt_2)_2$.

Introduction

Denitrification by anaerobic bacteria refers to the dissimilatory reduction of NO_3^- and NO_2^- to N_2O, which is further reduced to N_2.[1-3] These processes are of interest in biological removal of NO_3^- and NO_2^- from the water of rivers and lakes. Dissimilatory nitrate reductases contain iron, labile sulfide, and molybdenum[4] and have been purified from several bacteria. The dissimilatory nitrite reductases are divided into two types: one is a hemoprotein of cytochromes *c* and *d*[5-9] and the other is a copper-containing metalloflavoprotein.[10-12] Electrons required in the reduction of NO_3^- and NO_2^- by these reductases can be supplied from formate dehydrogenases, which oxidize HCOOH to CO_2.[13,16] The formate dehydrogenases coupled with those reductases are known to involve iron, labile sulfide, selenium, and molybdenum[17]

Previous model studies of nitrate reductases have shown that the reactions of NO_3^- with oxomolybdenum complexes give a variety of products such as NO_2, NO_2^-, NO, N_2O, NH_3OH^+, and NH_4^+, depending on the reaction conditions.[18-25] It has also been shown that NO_2^-, the first reduction product of NO_3^- by nitrate reductases, can be reduced to NO by oxomolybdenum complexes.[26] Catalytic reductions of NO_3^- and NO_2^- by oxomolybdenum complexes have, however, been unsuccessful so far. This paper demonstrates the first catalytic reduction of NO_2^- to N_2O by HCOOH in the presence of $MoO_2(S_2CNEt_2)_2$ or $MoO(S_2CNEt_2)_2$.

Experimental Section

Materials. Commercially available guaranteed reagent grade $NaNO_3$, $NaNO_2$, and HCOOH were used without further purification. Tetrabutylammonium nitrate and nitrite were prepare by the reactions of $NaNO_3$ and $NaNO_2$ with *n*-Bu_4NBr, respectively, in *N*,*N*-dimethylformamide (DMF). $MoO_2(S_2CNEt_2)_2$,[27] $MoO(S_2CNEt_2)_2$,[28] and $Na_2N_2O_2$[29] were prepared according to the literature methods. Solvent DMF was purified by distillation over CaO under reduced pressure of N_2, stored under dry nitrogen, and bubbled with He for at least 1 h right before use. Standard N_2O and CO_2 gases used for the quantitative analysis of reaction products were obtained from Gaschro Kogyo Co. Ltd.

Reactions. A rubber-septum-capped reaction flask (35 cm^3) containing $MoO_2(S_2CNEt_2)_2$ or $MoO(S_2CNEt_2)_2$ ($7.5 \sim 30.0$ μmol) was throughly flushed with He to remove air. A DMF (10 cm^3) solution containing $NaNO_2$ (300–600 μmol) and HCOOH (300–600 μmol) was injected into the flask through the septum cap by syringe techniques, and the solution was stirred magnetically at a given reaction temperature (±0.2 °C). At fixed time intervals, 0.1-cm^3 portions of gas and the solution were sampled from the gaseous and the liquid phases, respectively, in the flask through the septum cap. The quantitative analysis of the reaction products (CO_2 and N_2O) in both phases was performed on a Shimazu gas chromatograph GC-7A with a 3-m column filled with Gaschropack 54 (Gaschro Kogyo Co. Ltd.) at 100 °C with use of He (40 cm^3/min) as a carrier gas. The amount of $Na_2N_2O_2$ produced in the

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