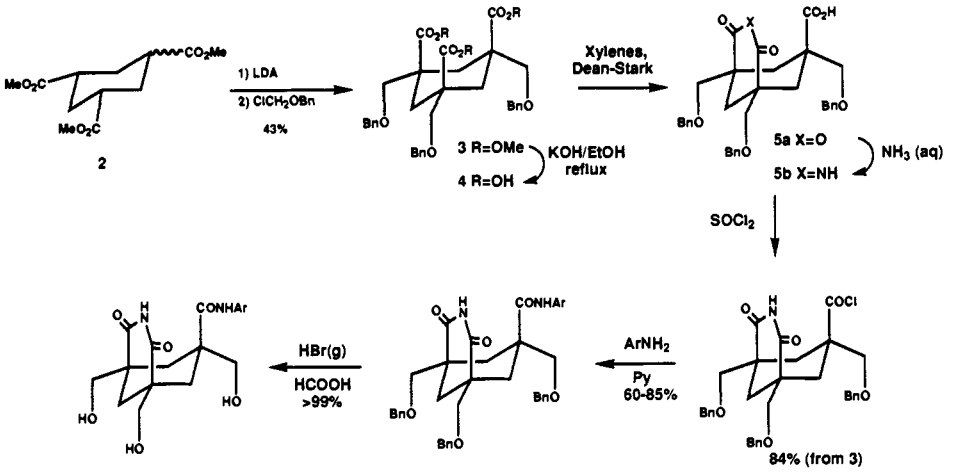
Scheme I



Scheme II

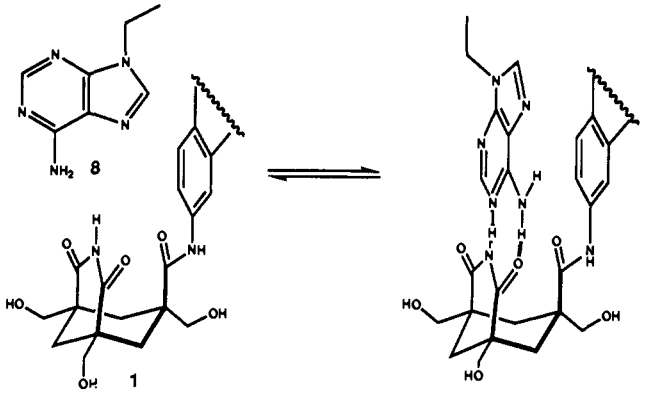
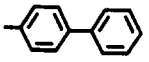
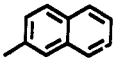
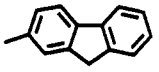
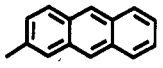


Table I. Solubilities of Hosts 1 in Water and Association Constants in Water with 9-Ethyladenine[a]

	Ar	solubility (mM)	K_a (M^{-1})
1a		15	2
1b		6	15
1c		1.2	29
1d		0.8	50
1e		0.2	70

[a] The solutions were buffered to a constant pH of 6.0 using 10 mM cacodylic acid/sodium cacodylate buffer (ionic strength 50 mM). NMR data were obtained at 283 K. Titrations were performed at a constant host concentration of 0.8 mM, except for **1e** where the concentration was 0.15 mM.[9]

1a has little overlap with the purine nucleus, and it provides a binding constant of 2 M^{-1}. Extension of the hydrophobic surface to the naphthyl host **1c** increases the association constant to 29 M^{-1}. This corresponds to a free energy change (ΔG) of −1.5 kcal/mol. While the relationship of surface area to hydrophobic binding is a matter of some uncertainty, our current results appear consistent with the values suggested by Honig.[10] The quantification of the smaller hydrogen-bonding contribution is the subject of current investigations.

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(10) Sharp, K. A.; Nicholls, A.; Fine, R. F.; Honig, B. *Science* **1991**, *252*, 106. For studies using other receptors, see: Smithrud, D.; Wyman, T.; Diederich, F. *J. Am. Chem. Soc.* **1989**, *113*, 5420. Petti, M.; Shepodd, T.; Barrans, R.; Dougherty, D. *J. Am. Chem. Soc.* **1988**, *110*, 6825. Cowart, M.; Sucholeiki, I.; Bukownik, R.; Wilcox, C. *J. Am. Chem. Soc.* **1988**, *110*, 6204.

Mechanism-Based Inactivation of Galactose Oxidase: Evidence for a Radical Mechanism

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Recently, substrate and protein radicals have been recognized as important intermediates in biological reactions.[1] Galactose oxidase (GOase) catalyzes the two-electron oxidation of primary alcohols with O_2 to produce aldehydes and H_2O_2.[2] GOase has two one-electron redox centers at the active site. GOase can exist in two stable forms: a one-electron-reduced inactive form and an oxidized active form.[3] Spectroscopic data show that the active form has Cu(II)[4] and another, non-metal, redox center at the active

(1) (a) Stubbe, J. *Biochemistry* **1988**, *27*, 3893. (b) Stubbe, J. *Annu. Rev. Biochem.* **1989**, *58*, 257-285. (c) Frey, P. A. *Chem. Rev.* **1990**, *90*, 1343. (d) Baldwin, J. E.; Bradley, M. *Chem. Rev.* **1990**, *90*, 1079. (e) Stubbe, J. *J. Biol. Chem.* **1990**, *265*, 5329. (f) Klinman, J. P.; Dooley, D. M.; Duine, J. A.; Knowles, P. F.; Mondovi, B.; Villafranca, J. J. *FEBS Lett.* **1991**, *282*, 1.

(2) (a) Kosman, D. J. In *Copper Proteins and Copper Enzymes*; Lontie, R., Ed.; CRC Press, Inc.: Boca Raton, FL, 1984; Vol. II, pp 1-26. (b) Hamilton, G. A. In *Copper Proteins. Metal Ions in Biology*; Spiro, T. G., Ed.; John Wiley & Sons: New York, 1981; Vol. 3, pp 193-218. (c) Ettinger, M. J.; Kosman, D. J. In *Copper Proteins. Metal Ions in Biology*; Spiro, T. G., Ed.; John Wiley & Sons: New York, 1981; Vol. 3, pp 219-261. (d) Tressel, P. S.; Kosman, D. J. *Methods Enzymol.* **1982**, *263*, 163.

(3) (a) Hamilton, G. A.; Adolf, P. K.; de Jersey, J.; DuBois, G. C.; Dyrkacz, G. R.; Libby, R. D. *J. Am. Chem. Soc.* **1978**, *100*, 1899. (b) Whittaker, M. M.; Whittaker, J. W. *J. Biol. Chem.* **1988**, *263*, 6074. (c) Babcock, G. T.; El-Deeb, M. K.; Sandusky, P. O.; Whittaker, M. M.; Whittaker, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3727.

(4) (a) Reference 3c. (b) Clark, K.; Penner-Hahn, J. E.; Whittaker, M. M.; Whittaker, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 6433. These results confirm an earlier report: (c) Blumberg, W. E.; Peisach, J.; Kosman, D. J.; Mason, H. S. In *Oxidases and Related Redox Systems*; King, T. E., Mason, H. S., Morrison, M., Eds.; Pergamon Press: Oxford, 1982; pp 207-224.

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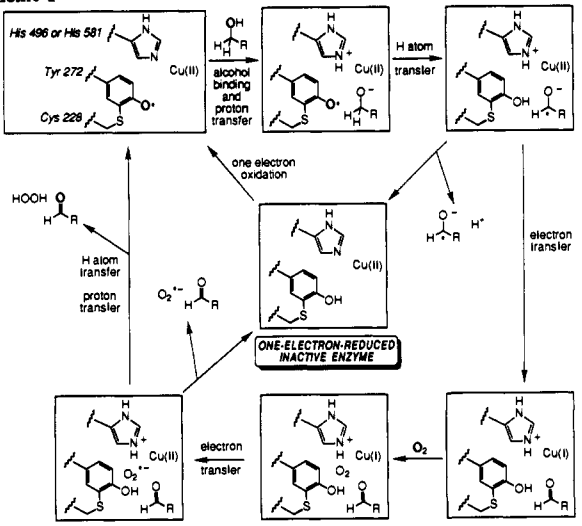
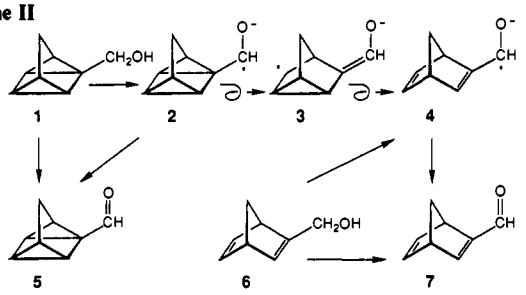
Scheme I



Scheme II



site.[5] X-ray structural determination indicates that this redox center is probably Tyr 272, a ligand to copper.[6] Tyr 272 is covalently cross-linked ortho to the OH to Cys 228 via an unusual thioether bond. GOase is not a pyrroloquinoline quinone containing enzyme, as has been suggested.[7] Prior to the discovery of the second redox site, several radical mechanisms were proposed using only copper as a redox site.[8] A new type of mechanism can be proposed using copper and a tyrosine as one-electron redox sites (Scheme I).[9] In this communication we present results consistent with this mechanism.

We examined **1** as a round-trip radical probe to attempt to detect the substrate-derived ketyl radical intermediate in the proposed mechanism (Scheme II).[10] Catalytic turnover of **1** could not be directly observed—O_2 electrode assays showed no O_2 uptake with as much as 500 mM **1**. GOase processing of **1** could be indirectly observed through mechanism-based inactivation (Figure 1). Inactivation kinetics was biphasic—for the fast phase k_{inact} = 0.025 s^{-1} and K_d = 36 mM.[11] The partition ratio, turnovers per inactivation event, must be nearly 1 (almost every molecule processed leads to inactivation) since no turnover was observed yet efficient inactivation occurred. The highly efficient inactivation made product analysis impractical, given the amounts of enzyme available. GOase was protected against inactivation by a standard substrate, β-methyl galactopyranoside, with K_d = 45 mM similar to its K_m of 47 mM,[12] indicating that inactivation occurs at the active site. Inactivations using [α,α-$^2H_2$]-**1** (CD_2OH) under saturating conditions (50 mM) had k_H/k_D on k_{inact} of 6.3. Standard substrates have k_H/k_D on k_{cat} of 6–8.[13] The inactivation does not require O_2 since **1** can inactivate GOase under anaerobic conditions.

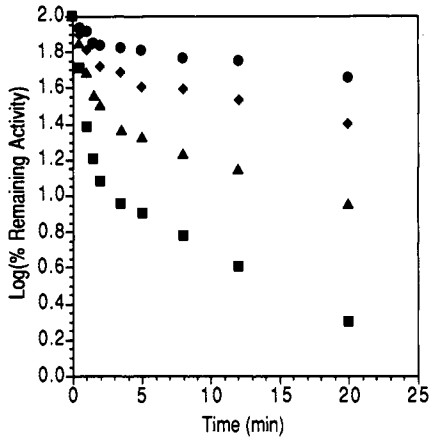


Figure 1. Inactivation of 75 nM GOase in air-saturated (0.24 mM O_2) 1:1 DMF/0.10 M (pH 7.0) sodium phosphate buffer at 25 °C with 5 (●), 10 (◆), 20 (▲), and 50 mM (■) **1**. At each time point aliquots were assayed using 100 mM β-methyl galactopyranoside as substrate in air-saturated 0.10 M (pH 7.0) sodium phosphate buffer at 25 °C. Control incubations without **1** did not cause inactivation (not shown on graph).

Table I. Oxidative Reactivation of GOase Inactivated by **1**

	relative % enzyme activity[a]	
treatment	native enzyme[b]	inactivated enzyme[c]
none	100	5 ± 3
$Fe(CN)_6^{3-}$	155 ± 7	150 ± 8
superoxide	270 ± 11	230 ± 13
superoxide + superoxide dismutase	120 ± 9	7 ± 4

[a] Determined with a YSI Clark type O_2 electrode using 100 mM β-methyl galactopyranoside as substrate in air-saturated 0.10 M (pH 7.0) sodium phosphate buffer at 25 °C. [b] Native enzyme was isolated as described in ref 2d. [c] Native enzyme was treated under air with 50 mM **1** for 2 h.

Highly efficient inactivation was also observed using **6** (partition ratio ~1). Inactivation kinetics was biphasic—for the fast phase k_{inact} = 0.015 s^{-1} and K_d = 0.07 mM. Inactivation could be completely reversed by one-electron oxidants. Inactivation by **6** indicates that inactivation with **1** is not due to any special feature of the quadricyclane ring in **1**.

All of these data are consistent with the mechanism shown in Scheme I involving rate-determining H-atom transfer from alcohol substrate to enzyme, generating a transient ketyl radical intermediate, followed by transfer of the second electron to generate

GOase inactivated by **1** could be completely reactivated using one-electron oxidants known to reactivate the one-electron-reduced inactive form (Table I). On this basis it can be concluded that mechanism-based inactivation of GOase using **1** produces the one-electron-reduced inactive form of GOase.[14]

(5) (a) Reference 3b. (b) Whittaker, M. M.; Whittaker, J. W. *J. Biol. Chem.* **1990**, *265*, 9610. (c) Whittaker, M. M.; DeVito, V. L.; Asher, S. A.; Whittaker, J. W. *J. Biol. Chem.* **1989**, *264*, 7104.

(6) Ito, N.; Phillips, S. E.; Stevens, C.; Ogel, Z. B.; McPherson, M. J.; Keen, J. N.; Yadav, K. D. S.; Knowles, P. F. *Nature* **1991**, *350*, 87.

(7) van der Meer, R. A.; Jongejan, J. A.; Duine, J. A. *J. Biol. Chem.* **1989**, *264*, 7792.

(8) (a) Reference 3a. (b) Driscol, J. J.; Kosman, D. K. *Biochemistry* **1987**, *26*, 3429. (c) Kwiatkowski, L. D.; Adelman, M.; Pennelly, R.; Kosman, D. J. *J. Inorg. Biochem.* **1981**, *14*, 209.

(9) This mechanism is a more detailed version of one which was previously proposed (see ref 3b) using an "organic" radical rather than the Tyr 272 radical.

(10) Branchaud, B. P.; Glenn, A. G.; Stiasny, H. C. *J. Org. Chem.* **1991**, *56*, 6656.

(11) Biphasic kinetics results from competing oxygen-independent substrate-induced inactivation of the oxidized active form and oxygen-dependent substrate-induced reactivation of the one-electron-reduced inactive form (unpublished results). Note that the inactivations level out to different steady states in a concentration-dependent fashion. Data are reported for only the first phase where the inactivation reaction predominates. The first phase is rapid and efficient—for example, 50 mM **1** leads to 88% loss of activity after 2 min.

(12) Clevland, L.; Coffman, R. E.; Coon, P.; Davis, L. *Biochemistry* **1975**, *14*, 1108.

(13) (a) Maradufu, A.; Cree, G. M.; Perlin, A. S. *Can. J. Chem.* **1971**, *49*, 3429. (b) Reference 2a.

(14) The one-electron-reduced form of GOase is known to be stable to air (O_2), whereas the two-electron-reduced form is not. Thus, substrate-inactivated GOase must be one-electron-reduced since it is stable to air. For the two-electron-reduced form, see ref 3b.

the aldehyde product. The inactivating substrates undergo the first "electron transfer" (H-atom transfer) but do not undergo the second electron transfer due to steric and/or stereoelectronic reasons, leaving the enzyme stalled in the one-electron-reduced inactive state.

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Reactions of Neutral Palladium Atoms in the Gas Phase: Formation of Stable Pd(alkane) Complexes at 300 K

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Some transition metals activate alkanes in low-energy conditions and others do not. For example, in the solution phase[1] coordinatively unsaturated Ru, Rh, Ir, and Pt centers insert into C–H bonds of alkanes, including methane. In the gas phase,[2] most of the 3d series bare metal cations Sc^+ through Ni^+ break C–H bonds or C–C bonds of propane and larger alkanes, leading to H_2 or CH_4 elimination products (or both). Thus far, only gas-phase cations attack C–C bonds of alkanes. Ta^+, W^+, Os^+, Ir^+, Pt^+, Nb^{2+}, Ta^{2+}, and Zr^{2+} dehydrogenate CH_4 at 300 K.[3,4] Neutral platinum and palladium clusters, Pt_x and Pd_x ($x \geq 2$), activate CH_4.[5] A key question is exactly how the electronic structure of the metal atom or metal center controls its chemical reactivity.[6] Gas-phase experiments in conjunction with high quality ab initio calculations[7-9] are beginning to show that the overall pattern of low-lying atomic states, including electron configuration and spin, strongly influences which metal atoms react and which do not.[10]

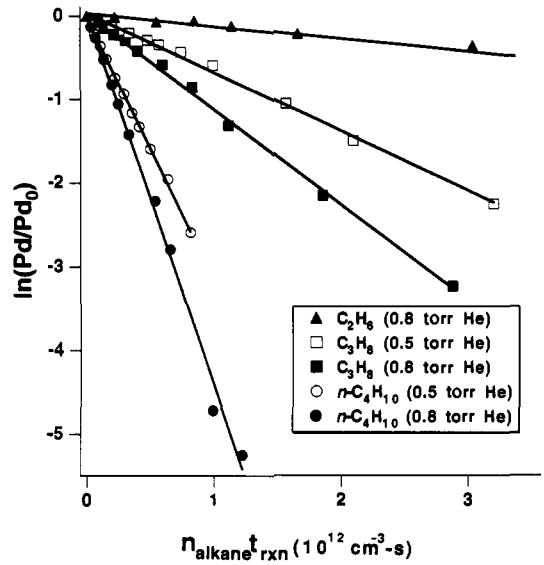

Figure 1. Semilogarithmic plots of Pd ($4d^{10}$, $^1S_0$) laser-induced fluorescence intensity vs the product of hydrocarbon number density and reaction time. Reactants and He pressures as indicated.

Table I. Effective Bimolecular Rate Constants (10^{-12} cm^3 s^{-1}) for Reactions of Pd and Ni with Alkanes and Alkenes at 300 K in He Buffer Gas[a]

	0.5 Torr of He		0.8 Torr of He	
reactant	Pd($4d^{10}$)	Ni($3d^94s$)[b]	Pd($4d^{10}$)	Ni($3d^94s$)[b]
C_2H_4	10.0 ± 1.0	0.5 ± 0.1	14.5 ± 1.5	0.5 ± 0.05
C_3H_6	-	12 ± 2	178 ± 18	11 ± 4
1-butene	314 ± 31	175 ± 35	357 ± 36	140 ± 30
CH_4	-	NR	NR	NR
C_2H_6	-	NR	0.15 ± 0.02	NR
C_3H_8	0.60 ± 0.06	NR	1.15 ± 0.12	NR
n-C_4H_{10}	3.1 ± 0.3	NR	4.4 ± 0.4	NR
cyclopropane	-	-	59 ± 6	10 ± 1

[a] NR means no reaction was observed ($k \leq 3 \times 10^{-14}$ cm^3 s^{-1}). Dash (-) means reaction was not studied. Ni data are from ref 12. [b] The reactivities of the Ni ground state $3d^84s^2(^3F_4)$ and the low-lying excited state $3d^94s^1(^3D_3)$ at 205 cm^{-1} were identical.

We and others[11] are surveying the gas-phase reactivity of bare neutral transition metal atoms with alkanes and alkenes. In the 3d series,[10,12] none of the neutral atoms reacts with alkanes at 300 K, and only Sc, Ti, V, and Ni react with alkenes. Here we report our first state-specific kinetics data for the 4d series. The $4d^{10}$ ($^1S_0$) ground state of the Pd atom reacts rapidly with ethylene, propylene, cyclopropane, and 1-butene and moderately rapidly with ethane, propane, and n-butane at 300 ± 5 K in 0.5–0.8 Torr of He. The latter are apparently the first clear-cut examples of gas-phase chemistry between a neutral ground-state transition metal atom and an alkane. Earlier gas-phase studies of Pd reactivity with CH_4 found no reaction of the Pd atom and very slow reaction of the clusters Pd_x, $x \geq 2$.[5c] CH_4 is inert to Rh atoms[5d] and to Fe atoms.[5e] The ground-state Pt atom *might* react with CH_4, n-hexane, and cyclohexane,[5a,5b] but interpretation was complicated by the possible presence of excited-state atoms or of multiphoton ionization and fragmentation.

The pressure dependence of our effective bimolecular rate constants indicates that the products are collisionally stabilized Pd(hydrocarbon) adducts.[13] The most likely structure of the

(1) (a) Janowicz, A. H.; Bergman, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 352. (b) Janowicz, A. H.; Bergman, R. G. *J. Am. Chem. Soc.* **1983**, *105*, 3929. (c) Hoyano, J. D.; Graham, W. A. G. *J. Am. Chem. Soc.* **1982**, *104*, 3723. (d) Hoyano, J. D.; McMaster, A. D.; Graham, W. A. G. *J. Am. Chem. Soc.* **1983**, *105*, 7190. (d) *Perspectives in the Selective Activation of C–H and C–C Bonds in Saturated Hydrocarbons*; Meunier, B., Chaudret, B., Eds.; Scientific Affairs Division-NATO: Brussels, 1988.

(2) (a) For a comprehensive review of M^+ chemistry, see: Eller, K.; Schwarz, H. *Chem. Rev.* **1991**, *91*, 1121. (b) For a review of M^{2+} chemistry, see: Roth, L. M.; Freiser, B. S. *Mass Spectrom. Rev.* **1991**, *10*, 303.

(3) Irikura, K. K.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 2769.

(4) (a) Buckner, S. W.; Gord, J. R.; Freiser, B. S. *J. Chem. Phys.* **1989**, *91*, 7530. (b) Ranasinghe, Y.; MacMahon, T. J.; Freiser, B. S. *J. Phys. Chem.* **1991**, 7721.

(5) Pt clusters: (a) Trevor, D. J.; Cox, D. M.; Kaldor, A. *J. Am. Chem. Soc.* **1990**, *112*, 3742. (b) Trevor, D. J.; Whetten, R. L.; Cox, D. M.; Kaldor, A. *J. Am. Chem. Soc.* **1985**, *107*, 518. Pd clusters: (c) Fayet, P.; Kaldor, A.; Cox, D. M. *J. Chem. Phys.* **1990**, *92*, 254. Rh clusters: (d) Zakin, M. R.; Cox, D. M.; Kaldor, A. *J. Chem. Phys.* **1988**, *89*, 1201. Fe clusters: (e) Whetten, R. L.; Trevor, D. J.; Cox, D. M.; Kaldor, A. *J. Phys. Chem.* **1985**, *89*, 566.

(6) (a) Weisshaar, J. C. In *State-Selected and State-to-State Ion-Molecule Reaction Dynamics*; Ng, C.-Y., Ed.; Wiley: New York, 1992; Part I. (b) Armentrout, P. B. *Annu. Rev. Phys. Chem.* **1990**, *41*, 313.

(7) See, for example: (a) Blomberg, M. R. A.; Siegbahn, P. E. M.; Nagashima, U.; Wennerberg, J. *J. Am. Chem. Soc.* **1991**, *113*, 424. (b) Blomberg, M. R. A.; Siegbahn, P. E. M.; Svensson, M. *J. Phys. Chem.* **1991**, *95*, 4313. (c) Blomberg, M. R. A.; Siegbahn, P. E. M.; Svensson, M. *J. Am. Chem. Soc.* **1992**, *114*, 6095.

(8) Low, J. J.; Goddard, W. A., III *J. Am. Chem. Soc.* **1984**, *106*, 8321.

(9) Langhoff, S. R.; Bauschlicher, C. W., Jr. *Annu. Rev. Phys. Chem.* **1988**, *39*, 181.

(10) Weisshaar, J. C. In *Gas Phase Metal Reactions*; Fontijn, A., Ed.; ACS Symposium Series; Elsevier: Amsterdam, 1992.

(11) (a) Mitchell, S. A. In *Gas Phase Metal Reactions*; Fontijn, A., Ed.; ACS Symposium Series; Elsevier: Amsterdam, 1992. (b) Parnis, J. M.; Mitchell, S. A.; Hackett, P. *J. Phys. Chem.* **1990**, *94*, 8152. (c) Mitchell, S. A.; Hackett, P. *J. Chem. Phys.* **1990**, *93*, 7822. (d) Blitz, M. A.; Mitchell, S. A.; Hackett, P. *J. Phys. Chem.* **1991**, *95*, 8719.

(12) (a) Ritter, D.; Weisshaar, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 6426. (b) Ritter, D.; Carroll, J.; Weisshaar, J. C. *J. Phys. Chem.*, in press.

(13) Observation of such adducts is commonplace in metal cation chemistry. See: Tonkyn, R.; Ronan, M.; Weisshaar, J. C. *J. Phys. Chem.* **1988**, *92*, 92.

0002-7863/93/1515-800$04.00/0