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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Methanolysis of Thioamide Promoted by a Simple Palladacycle Is Accelerated by 10^8 over the Methoxide-Catalyzed Reaction

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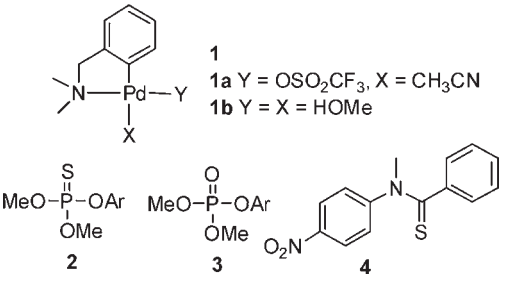
Supporting Information

ABSTRACT: Palladacycle **1** catalyzes the methanolytic cleavage of *N*-methyl-*N*-(4-nitrophenyl)thiobenzamide (**4**) via a mechanism involving formation of a Pd-bound tetrahedral intermediate (**TI**). The rate constant for decomposition of the complex formed between **1**, methoxide, and **4** is 9.3 s^{-1} at 25 °C; this reaction produces methyl thiobenzoate and *N*-methyl-4-nitroaniline. The ratio of the second-order rate constant for the catalyzed reaction, given as k_{cat}/K_d, relative to that of the methoxide-promoted reaction is 3×10^8, representing a very large catalysis of thioamide bond cleavage by a synthetic metal complex.

Thioamide-modified peptides are being investigated in search of more stable and potent compounds possessing useful bioactivity that is absent in normal peptide counterparts.[1,2] Thioamide-based natural products have recently been isolated from plants[3] and bacteria.[4,5] For example, closthioamide, found in the Gram-positive *Clostridium celluloyticum*, is a symmetric hexathioamide exhibiting very high antibacterial activity against methicillin-resistant *Staphylococcus aureus* as well as the vancomycin-resistant *Enterococcus faecalis* strains.[5] Relative to amides, thioamides show greater double-bond character between C and N[6,7] and are more resistant to solvolytic and enzymatic cleavage.[2,7−10] Understanding the mechanisms for solvolytic and metallocatalytic thioamide cleavage may provide insights into enzymatic reactions and aid further development of bioactive compounds. This is also of interest for understanding the modes of catalysis available to metallopeptidases[11] for cleavage of regular amides.

Earlier we demonstrated that palladacycle **1**, having a "soft"[12] Pd (II) ion, is an efficient catalyst for promoting the methanolytic cleavages of neutral phosphorothioates **2** but not those of their P═O counterparts **3**.[13] Although the "harder"[12] La^{3+} and Zn^{2+} ions are efficient catalysts for alcoholysis of phosphates, carboxylate esters,[14] and highly activated amides,[15] they do not appreciably catalyze the cleavage of less activated amides. This is most likely due to a catalytic requirement to provide both substrate activation and assistance with leaving group departure[15b] if the leaving group is poor. The catalytic cleavage of **2** involves transient sulfur binding to **1** (Y = HOMe, X = $^-OCH_3$) followed by intramolecular CH_3O^- attack on P to form a Pd-bound five- coordinate thiophosphorane intermediate, the decomposition of which yields trimethyl phosphorothioate and the corresponding phenol. The key to the catalytic reaction involves the interactions of the "soft" Pd and the thiophosphoryl group, which direct the initial substrate binding and subsequent formation of the Pd-bound thiophosphorane intermediate. Since the leaving groups in series **2** are good, there is no requirement to assist their departure from the phosphorane. However, one might expect that with a thioamide substrate, departure of the leaving group would be slow and thus require assistance by a softer metal ion such as Pd, whose hard/soft characteristics are more closely matched to those of a departing amide (amine).



Herein we describe the rapid methanolytic C−N cleavage of thioamide **4** in the presence of the palladacycle Pd(*N*,*N*-dimethylbenzylamine)(CH_3CN)(OSO_2CF_3) (**1a**), where these expectations are realized. (The CH_3CN and OSO_2CF_3 ligands of palladacycle **1a** are immediately replaced by solvent on dissolution in methanol.) We also show that the reaction is accelerated by 10^8 relative to the methoxide-promoted methanolysis. All of the evidence points to interactions of the Pd ion with the "soft" S═C unit to make the bound thioamide substrate more susceptible to intramolecular Pd−OCH_3 attack as well as Pd assistance of the subsequent the departure of the leaving group to produce the aniline and methyl thiobenzoate.

There are several observations of note. First, apparent saturation kinetics was observed for all the plots of k_{obs} versus [**1a**] with substrate **4** between ^s_spH 7.7 and 14.8.[16] Figure 1 shows a typical plot of k_{obs} for the formation of *N*-methyl-4-nitroaniline from the methanolysis of **4** versus [**1a**] in a 2 mM buffer of 1-ethylpiperidine at ^s_spH 10.6. The data were fit to an equation applicable to both strong and weak binding situations[14] [see the Supporting Information (SI)] to give a dissociation constant (K_d) of $10^{-(4.89\pm0.05)}$ M for the **1**:**4** complex and a k_{cat} of 0.025 s^{-1} for its cleavage. The production of *N*-methyl-4-nitroaniline was confirmed by comparing the UV−vis and ^{1}H NMR spectra of the product mixture with those of an authentic mixture.

The kinetic parameters (K_d and k_{cat}) obtained from individual plots of k_{obs} versus [**1a**] (see the SI) at various ^s_spH are given in Table 1. The K_d values are the same within the experimental

Received: September 1, 2011
Published: November 16, 2011

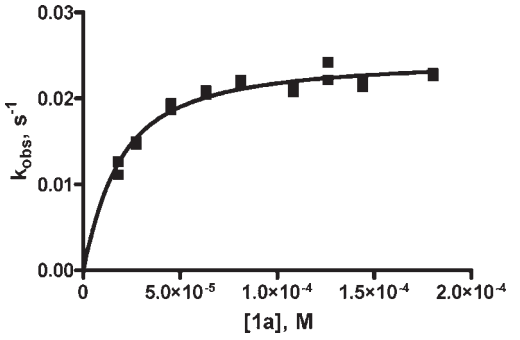


Figure 1. Plot of k_{obs} for the methanolysis of **4** (0.01 mM) vs [**1a**] at 25 °C, ${}^{s}_{s}$pH 10.6 ± 0.1 under buffered conditions in 2 mM 1-ethylpiperidine. The data were fit to eq 1S in the SI to obtain $K_d = 10^{-(4.89\pm0.05)}$ M and k_{cat} = 0.0248 ± 0.0005 s^{-1}.

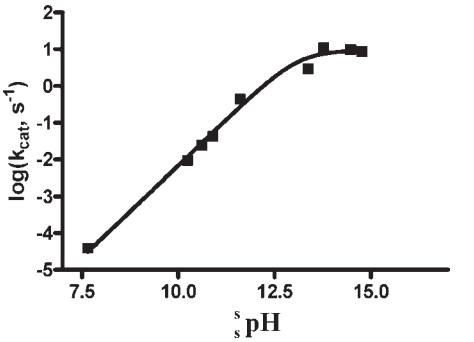


Figure 2. Plot of log k_{cat} vs ${}^{s}_{s}$pH for the **1a**-catalyzed cleavage of **4** in MeOH at 25 °C. The data were fit to eq 1 to give a kinetic ${}^{s}_{s}pK_a$ of 13.1 ± 0.1 and k_{max} = 9.3 ± 2.1 s^{-1} (r^2 = 0.9926).

Table 1. k_{cat} and K_d Values Obtained for the 1a-Promoted Methanolysis of 4 at Various ${}^{s}_{s}$pH in MeOH at 25 °C

${}^{s}_{s}$pH	k_{cat} (s^{-1})	−log(K_d/M)
7.7 ± 0.1	(3.9 ± 0.2) × 10^{-5}	4.9 ± 0.2
10.2 ± 0.1	(9.7 ± 0.2) × 10^{-3}	5.00 ± 0.05
10.5 ± 0.1[a]	(2.0 ± 0.2) × 10^{-2} [b]	
10.6 ± 0.1	(2.48 ± 0.05) × 10^{-2}	4.89 ± 0.05
10.9 ± 0.1	(4.32 ± 0.09) × 10^{-2}	5.23 ± 0.07
11.6 ± 0.1	(4.2 ± 0.1) × 10^{-1}	5.3 ± 0.1
13.37[a]	3.01 ± 0.03 [b]	[c]
13.77[a]	11.2 ± 0.5 [b]	[c]
14.47[a]	10.7 ± 0.3	[c]
14.77[a]	8.8 ± 0.2 [b]	[c]

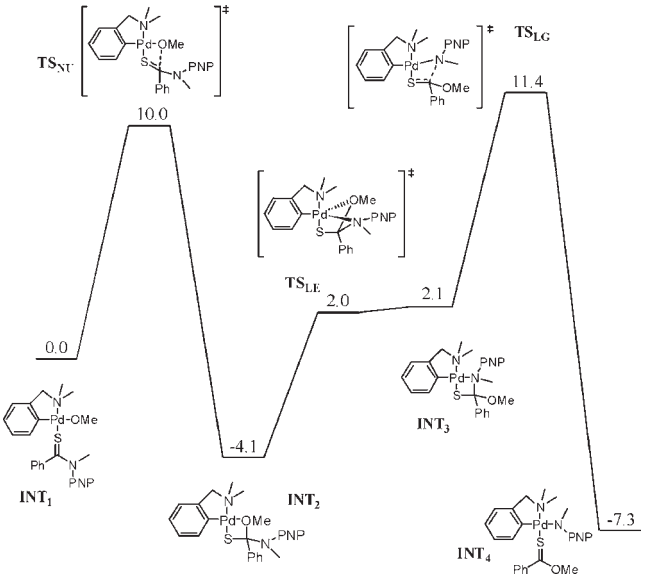
[a] Obtained using a pH-jump method; the ${}^{s}_{s}$pH values were calculated as ${}^{s}_{s}$pH = 16.77 + log[NaOMe], where $10^{-16.77}$ M^2 is the autoprotolysis constant of MeOH.[16] [b] Obtained using 0.01 mM **4** and 0.15 mM **1a**, conditions where the catalyst and the substrate are fully bound. [c] Experimental binding constants could not be obtained because of the use of the pH-jump method (see the text).

uncertainty and suggest strong binding between **1** and **4** at all ${}^{s}_{s}$pH values up to 11.6. Between ${}^{s}_{s}$pH 11.6 and 14.77, NaOMe was used to keep [$^{-}$OMe] constant. The kinetic method for these experiments involved a stopped-flow, pH-jump method (described later) in which a preformed **1**:**4** complex was rapidly mixed with NaOMe, deprotonating the Pd-bound HOMe with an assumed diffusion-limited rate constant. The data in Table 1 (Figure 2) were numerically fit to eq 1, which was developed for a process controlled by a single ionization, to give a kinetic ${}^{s}_{s}pK_a$ of 13.1 ± 0.1 and k_{max}= 9.3 ± 2.1 s^{-1}.

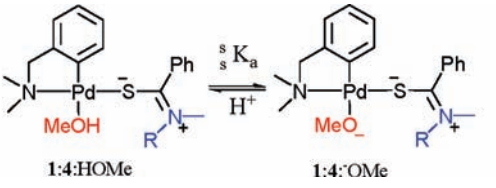
$$k_{cat} = \frac{k_{max} \bullet {}^{s}_{s}K_a}{{}^{s}_{s}K_a + [H^+]} \quad (1)$$

The kinetic ${}^{s}_{s}pK_a$ of 13.1 for the ionization of **1**:**4**:HOMe (illustrated in eq 2 below) is larger than the reported value of 10.8−11.0 for ionization of **1** (X = HOMe; Y = pyridine).[13] The elevation in the ${}^{s}_{s}pK_a$ of the Pd-bound methanol when the palladacycle is complexed to **4** is consistent with the thioamide being represented as a dominant resonance form with the S atom having substantial negative charge[6a] that lowers the Lewis acidity of the Pd. It has been shown previously that when Pd is bound to

Scheme 1. DFT-Computed Reaction Pathway for the 1-Catalyzed Methanolysis of 4 [the ΔG Values for Intermediate Structures and Transition States (in kcal/mol) Are to Scale]



an anionic phosphorothioate diester (with P−S^{-}), the ${}^{s}_{s}pK_a$ of the Pd-bound methanol is 12.7 ± 0.2.[17]



(2)

The steps of the **1**-catalyzed methanolysis of **4** were modeled using density functional theory (DFT) calculations[18−22] that revealed additional mechanistic insights. The lowest-energy pathway proceeding from **1**:**4**:$^{-}$OMe is shown in Scheme 1. It is important to note that the plateau region of Figure 2 was probed experimentally using a pH-jump procedure in which preformed **1**:**4**:HOMe was instantly converted to **1**:**4**:$^{-}$OMe. The latter follows a low-energy pathway to product and under the basic conditions is not in equilibrium with a substrate-dissociated form,

dx.doi.org/10.1021/ja209605r |J. Am. Chem. Soc. 2011, 133, 20068–20071

since the barrier for methanol replacement of **4** from **1**:**4**:$^{-}$OMe is larger than the barriers for proceeding to product. The initial structure modeled ($\mathbf{INT_1}$, corresponding to **1**:**4**:$^{-}$OMe in eq 2) involves the substrate bound trans to the amine of the square-planar palladacycle as the instantaneously formed deprotonation product of the most thermodynamically stable **1**:**4**:HOMe species (see the SI). Nucleophilic attack of the Pd-bound methoxide ($\mathbf{TS_{Nu}}$) occurs with a low barrier of $\Delta G^{\ddagger}$ = 10.0 kcal mol^{-1}, leading to a relatively stable tetrahedral intermediate ($\mathbf{INT_2}$) with both S and nucleophile OMe coordinated to the Pd in a four-membered ring. Departure of the leaving group (LG) is assisted by binding of the N(CH$_3$)−PNP to Pd, which occurs through a process involving replacement of the metal-bound methoxide with the LG nitrogen. This was modeled as a direct ligand exchange via a trigonal-bipyramidal transition state ($\mathbf{TS_{LE}}$). The subsequent Pd-bound LG intermediate, $\mathbf{INT_3}$, is energetically indistinguishable from $\mathbf{TS_{LE}}$ and likely is short-lived and exists transiently prior to LG expulsion. The LG departs through a lengthening of the C−N bond with the Pd−N bond remaining intact ($\mathbf{TS_{LG}}$), and this TS is associated with a free energy 11.4 kcal mol^{-1} relative to $\mathbf{INT_1}$ (or 15.5 kcal mol^{-1} relative to $\mathbf{INT_2}$).

The step from $\mathbf{INT_1}$ to formation of the Pd-bound tetrahedral intermediate ($\mathbf{INT_2}$) is similar to what has been demonstrated for the **1**-promoted methanolyses of phosphorothioate triesters **2**[13] and proposed for cleavage of the analogous diesters.[17] However, the rate-limiting step in the case of the cleavage of thioamides involves expulsion of a far poorer leaving group than the aryloxides in **2**, namely, the corresponding anilide of **4**. It has been shown in numerous cases[23,24] that the rate-limiting step during the alkaline methanolysis or hydrolysis of amides is C−N$_{LG}$ bond cleavage. Solvolytically, this generally involves a transition state where the C−N$_{LG}$ bond cleavage is coupled with solvent-assisted departure (through protonation) to form an aniline or amine. However, in the case of the palladacycle-catalyzed cleavage of **4**, the solvent kinetic isotope effect ($k_{cat}^{MeOH}/k_{cat}^{MeOD}$) of 1.20 ± 0.04 determined in the plateau region of Figure 2 suggests that there is no proton in flight, as would be expected if the solvent were to act as a general acid. Furthermore, the absence of buffer catalysis in the ascending wing of Figure 2 indicates that protonated buffer does not act as a general acid to facilitate the departure of the leaving group.

In support of the general mechanism given in Scheme 1, with the rate-limiting step being the Pd-assisted departure of the anilide from $\mathbf{INT_3}$, we note that the experimental activation parameters determined in the plateau region of Figure 2 are $\Delta H^{\ddagger}$ = 16.4 ± 0.4 kcal/mol, $\Delta S^{\ddagger}$ = 0.7 ± 1.3 cal K^{-1} mol^{-1}, and $\Delta G^{\ddagger}_{298K}$ = 16.2 kcal/mol. These can be compared with the values $\Delta H^{\ddagger}$ = 19.9 kcal/mol and $\Delta S^{\ddagger}$ = −11 cal K^{-1} mol^{-1} reported for the basic methanolysis of **4**,[10] $\Delta G^{\ddagger}_{298K}$ = 22.2 kcal/mol.[25] The experimental $\Delta S^{\ddagger}$ value of 0.7 cal K^{-1} mol^{-1} found for the palladacycle-promoted reaction can be compared with the computed ΔS values of −11 cal K^{-1} mol^{-1} for passing from $\mathbf{INT_1}$ to $\mathbf{TS_{LG}}$ and −3 cal K^{-1} mol^{-1} for passing from $\mathbf{INT_2}$ to $\mathbf{TS_{LG}}$ (see the SI). The latter value, coupled with a computed $\Delta G^{\ddagger}$ of 15.5 kcal/mol for passing from $\mathbf{INT_2}$ to $\mathbf{TS_{LG}}$, agree remarkably well with the experimentally observed values. This in turn may suggest that the observable process deals with the breakdown of the Pd-bound tetrahedral intermediate $\mathbf{INT_2}$ via metal-ion-assisted departure of the leaving group as in $\mathbf{INT_3}$.[7]

The acceleration provided by **1** for cleavage of **4** is large in comparison with that for the methoxide-promoted reaction. The k_2^{OMe} value is (1.06 ± 0.03) × 10^{-4} M^{-1} s^{-1} at 25 °C (see the SI). However, the apparent second-order rate constant for methoxide attack on the **1**:**4** complex is k_{app}^{OMe} = 3.6 × 10^4 M^{-1} s^{-1}, as determined from the linear part of the $^{s}_{s}$pH−rate constant profile in Figure 2, providing an acceleration of 3.4 × 10^8 relative to methoxide attack on the free substrate.[26] This value is the highest known for a metal-ion-promoted solvolysis reaction of a thioamide and compares favorably to what is seen for hydroxide-promoted cleavage of some intramolecularly tethered Cu^{2+}-bound amides.[28]

Palladacycle **1a** does not appreciably catalyze the methanolysis of the analogous C═O-containing amide, *N*-methyl-*N*-(4-nitrophenyl)benzamide. This is a probable consequence of the poor interaction between the "soft" Pd and the "hard" C═O unit, which precludes the first required step of the catalytic cycle, namely, bringing the substrate and catalyst productively together in an orientation that allows the subsequent steps to occur. This brings to mind the results of a study involving native carboxypeptidase A, a zinc-containing digestive peptidase, that catalyzes C−N bond cleavage of C═O-containing peptides with k_2 ($=k_{cat}/K_M$) values of ∼10^5−10^6 M^{-1} s^{-1}.[7−9,11c,27] The native enzyme catalyzes the cleavage of analogous thioamide-containing peptides 100−1000 times more slowly,[7−9] probably because of the mismatch of the hard−soft characteristics of the metal ion and the substrate. However, replacing the native Zn(II) ion with the softer and more thiophilic Cd(II) ion results in enhanced enzyme activity toward the thioamide peptides while hindering the cleavage of normal amides.[9]

In summary, the Pd in **1a** clearly plays multiple catalytic roles in the reaction described herein, including enhancing the electrophilicity of the thioamide through favorable substrate binding and orientation for intramolecular attack of a Pd-bound nucleophile. It is an interesting prediction of the computational study that palladacycle binding to the tetrahedral intermediate makes $\mathbf{INT_2}$ lie lower in energy than $\mathbf{INT_1}$, suggesting that it might be possible to observe this intermediate under suitable conditions, similar to what we have observed in the case of Pd-promoted methanolysis of phosphorothioate triesters.[13b] Moreover, the available evidence indicates that the Pd provides the required assistance with LG departure via a process predicted to involve transient association with N in a second tetrahedral intermediate or transition structure termed $\mathbf{INT_3}$.

More work is required to delineate all of the features of this large rate acceleration of thioamide solvolysis. Further results of comprehensive investigations of the palladacycle-catalyzed solvolysis of a series of different thioamides, the effects of different solvents including water, and extensive DFT studies will be disclosed in due course.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details, original kinetic data, titration data, UV−vis spectra, DFT calculation details, and complete ref 22. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support of NSERC Canada and Queen's University. S.G.P. acknowledges the support of an NSERC USRA (May−August 2011).

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(18) Optimizations and free energy determinations of ground and transition states were calculated at the B3LYP level[19] with the IEFPCM[20] methanol solvent model applied. The 6-31G(d,p) basis set was used for C and H and the 6-31++G(d,p) basis set for heteroatoms. The effective core potential of Hay and Wadt[21] with the double-ζ valence basis set (LANL2DZ) was used to describe Pd. Frequency calculations were used to characterize minima (intermediates) and maxima (transition states) on the potential energy surface and as a basis for free energy determinations at 298 K. All of the calculations were conducted using Gaussian 09.[22]

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(26) An alternative method for determining the acceleration of the catalytic reaction relative to the methoxide-promoted reaction involves comparison of the second-order rate constant for the attack of **1**:$^-$OMe with that of free methoxide on the unbound substrate. This can be calculated as $(k_{cat}/K_d)[K_a/(K_a + H^+)]$ at a pH below that of the **1**:HOMe ionization. When this was calculated at pH 7.7, the **1**:$^-$OMe form was found to be 6×10^7 times more reactive than free methoxide.

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