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Communications

Transaminative Desilylation of (Aminomethyl)trimethylsilane and Transitory Inactivation of Plasma Amine Oxidase[1]

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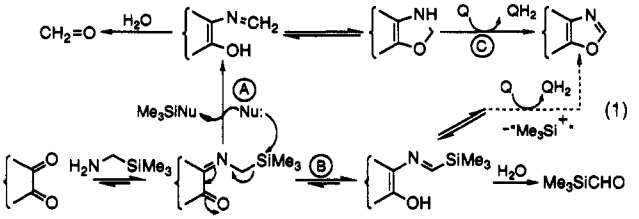
Summary: (Aminomethyl)trimethylsilane (AMTMS), a compound which has previously been used as a probe for electron-transfer oxidation of amines, undergoes transaminative desilylation by active carbonyl reagents and induces a mechanism-based inactivation of the quinone-containing plasma amine oxidase.

(Aminomethyl)trimethylsilane (AMTMS) was reported by Banik and Silverman to induce a slowly recoverable, mechanism-based inactivation of the mitochondrial flavin-dependent monoamine oxidase (MAO),[2] an enzyme believed to oxidize amines via initial one-electron (1e) oxidation at nitrogen. The interest in AMTMS stemmed from Mariano's earlier demonstration that photochemical 1e oxidation of α-heteroatom-substituted silanes labilizes the C–Si bond.[3,4] Nonetheless, AMTMS can be viewed as an analog of glycine wherein one potential C_α electrofuge (CO_2) is replaced by another (Me_3Si^+). We thus anticipated that the C–Si bond in AMTMS would also be labilized upon a formal *two-electron* oxidation at nitrogen, vis-a-vis pyridoxal phosphate (PLP)-mediated decarboxylation of amino acids. In fact, the benzaldehyde imine of AMTMS has been used as an azomethine ylide synthon.[5] We herein provide several examples of chemical transaminative desilylation of AMTMS by active carbonyl reagents. These findings led us to consider AMTMS as a potential probe of mechanism for primary amine deamination catalyzed by the quinone-containing copper amine oxidases,[6] and we present data which suggest AMTMS is a mechanism-based inactivator of bovine plasma amine oxidase (BPAO).

Chemistry. Using a 1,2-dicarbonyl grouping as a specific example of an active carbonyl reagent, eq 1 illustrates



how Schiff base formation with AMTMS can labilize the C–Si bond (path A) as well as facilitate normal transamination (path B). We examined the reaction of AMTMS with both pyridoxal and isatin, which, although possessing weak transaminating power, have been com-

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(6) The cofactor for the entire enzyme class was believed for some time to be pyrroloquinoline quinone (PQQ),[7] but recent studies demonstrated that the cofactor quinone is a posttranslationally modified active-site aromatic amino acid. (a) 2,4,5-Trihydroxyphenylalanine (TOPA) quinone in the case of BPAO: Janes, S. M.; Mu, D.; Wemmer, D.; Smith, A. J.; Kaur, S.; Maltby, D.; Burlingame, A. L.; Klinman, J. P. *Science* **1990**, *248*, 981. Brown, D. E.; McGuirl, M. A.; Dooley, D. M.; Janes, S. M.; Mu, D.; Klinman, J. P. *J. Biol. Chem.* **1991**, *266*, 4049. (b) Tryptophan tryptophylquinone in the case of methylamine dehydrogenase: McIntire, W. S.; Wemmer, D. E.; Chistoserdov, A.; Lidstrom, M. E. *Science* **1991**, *252*, 817. Chen, L.; Mathews, F. S.; Davidson, V. L.; Huizinga, E. G.; Vellieux, F. M. D.; Duine, J. A.; Hol, W. G. J. *FEBS Lett.* **1991**, *287*, 163.

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monly employed as PLP models.[8] In CH_3CN–H_2O (1:1, v/v) at pH 8 (0.15 M carbonate), 1 equiv of pyridoxal (70 °C, 3 days, closed pressure tube) or isatin (25 °C, 24 h) induced oxidative cleavage of AMTMS, generating CH_2=O (isolated as the 2,4-dinitrophenylhydrazone) in 20% and 51% yield, respectively, and, in part, the corresponding reductive amination products (pyridoxamine and 3-amino-2-oxindole).

As models for the quinone cofactor of copper amine oxidases,[6] we previously employed 3,5-di-*tert*-butyl-1,2-benzoquinone (*o*-DTBQ)[9] and 2,6-di-*tert*-butyl-1,4-benzoquinone (*p*-DTBQ), where the *tert*-butyl groups sterically block the Michael addition pathway which would otherwise supercede the carbonyl condensation pathway. *o*-DTBQ is by far the stronger transaminating agent and was originally introduced by Corey for effecting preparatively useful conversions of *branched* primary amines to ketones,[10] though *unbranched* primary amines lead primarily to benzoxazoles, arising from quinone oxidation of the cyclic hemiaminal tautomers of the initially formed aminophenol Schiff bases (see, e.g., eq 1, path C). In fact, we found 1 equiv of *o*-DTBQ to induce rapid oxidative desilylation of AMTMS in CH_3CN–H_2O (1:1) at 25 °C, pH 7, generating 5,7-di-*tert*-butylbenzoxazole[11] in 41% (N_2, maximal yield 50%) or 68% (air) isolated yield. Under excess amine conditions, the benzoxazole-forming (path C) reaction is effectively suppressed: using 1.25 mM *o*-DTBQ and 10 mM AMTMS in CH_3CN–H_2O (1:1) at 25 °C, 0.1 M pH 7 phosphate buffer, under N_2, we obtained a 70% yield of CH_2=O (as its 2,4-dinitrophenylhydrazone) based on *o*-DTBQ. Pseudo-first-order plots of the rapid quinone consumption under these conditions exhibited linear behavior (k = 0.17 and 0.99 min^{-1} at pH 7 and 8) after a lag period (ca. 200 and 100 s at pH 7 and 8) associated with a diminished slope, indicative of the expected preliminary buildup of quinoneimine intermediate.[12]

In order to avert the path C complication for a quinone–amine 1:1 stoichiometry, we resorted to using *p*-DTBQ, which is however a substantially weaker transaminating agent and was found to effect cleavage of AMTMS in CH_3CN–H_2O at 75 °C (closed pressure tube), pH 8 (carbonate), N_2, in 3 days, affording CH_2=O in 48% yield. The expected product of reductive amination in this case is 2,6-di-*tert*-butyl-4-aminophenol, a material known to be extremely sensitive to autoxidation.[13,14] We confirmed by TLC and ^1H NMR that the organic product of the *p*-DTBQ–AMTMS reaction was the same mixture, containing mainly 2,3′,5′,6-tetra-*tert*-butylindophenol,[14,15] as that obtained from decomposition of the independently synthesized[13] aminophenol. Although we have not attempted to optimize any of the above reactions, the potential for transaminative desilylation of AMTMS is clear.

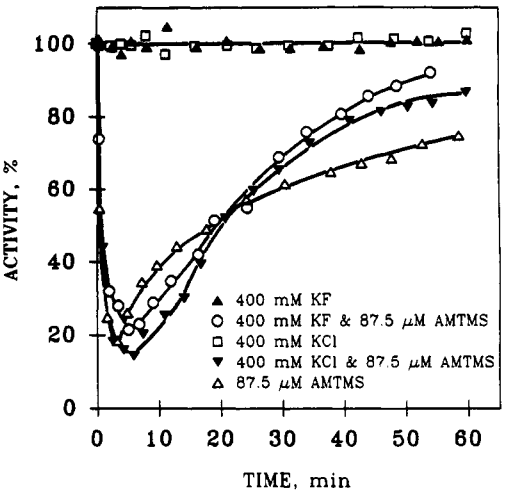

Figure 1. Effect of KF and KCl on the inactivation of BPAO by AMTMS in pH 9.0 tris buffer (50 mM), 30 °C.

Enzymology. On the basis of the above chemistry, we envisioned that processing of AMTMS by the quinone cofactor of copper amine oxidases might result either in transaminative desilylation and possibly attachment of Me_3Si^+ to an active site nucleophile (eq 1, path A) or in simple transamination (path B), which after hydrolysis would produce Me_3SiCH=O, a potential alkylating agent.[2] We found AMTMS to produce a rapid inactivation of commercial BPAO;[16] studies at low temperature were needed to verify the time dependence of activity loss at higher AMTMS concentrations. Kitz and Wilson replots ($t_{1/2}$ vs 1/[AMTMS])[18] of the inactivation data did not provide convincing evidence for saturation (the deviation from intersection at the 0–0 origin is not statistically significant), and the data can be satisfactorily evaluated at present in terms of bimolecular inactivation kinetics (k_i = 70, 146, and 270 M^{-1} s^{-1} at 5, 15, and 30 °C).

The specific substrate benzylamine protected against inactivation in a concentration-dependent manner.[19] Furthermore, an enzyme preparation which had been rendered 97% inactive by incubation for 30 min with 0.4 mM AMTMS remained 92% inactive following gel chromatography (sephadex G-25) performed within 15 min. A plot of activity vs enzyme concentration (in units/mL) in the absence and presence of constant [AMTMS] = 40 μM gave two parallel lines, revealing a titration point (30 °C, 30 min incubation) at 0.0155 units/mL using 40 μM AMTMS. On the basis of a specific activity of 0.48 units/mg for the "pure" dimeric enzyme (two catalytic 85 kD subunits),[17] our measurement corresponds to an apparent partition ratio of ~105, suggesting that substantial turnover of AMTMS accompanies enzyme inactivation. We did detect generation of CH_2=O, the expected desilylation product, though additional studies are needed to quantify it and/or the product(s) of normal turnover.

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(11) ^1H NMR ($CDCl_3$) δ 1.41 (s, 9 H), 1.51 (s, 9 H), 7.34 and 7.68 (2d, 1 H each, J = 1.8 Hz), 8.10 (s, 1 H). This compound has been previously reported: Haeussler, H.; Jadamus, H. *Chem. Ber.* **1964**, *97*, 3051.

(12) The observed biphasic kinetics and isolation of benzoxazole when using a 1:1 stoichiometry demonstrates that the reaction between *o*-DTBQ and AMTMS does not involve initial electron transfer.[9] In contrast, the oxidation of *tertiary* amines by *strong* quinone oxidants such as chloranil and DDQ may involve electron transfer: Buckley, D.; Dunstan, S.; Henbest, H. B. *J. Chem. Soc.* **1957**, 4880. Goswami, A.; Schaumberg, J. P.; Duffel, M. W.; Rosazza, J. P. *J. Org. Chem.* **1987**, *52*, 1500.

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(14) Coppinger, G. M. *Tetrahedron* **1962**, *18*, 61.

(15) ^1H NMR ($CDCl_3$) δ 1.26 (s, 9 H), 1.34 (s, 9 H), 1.46 (s, 18 H), 6.85 (s, 2 H), 7.04 and 7.06 (2d, 1 H each, J = 2.6 Hz).

(16) Enzyme studies were conducted at pH 7.2 (50 mM phosphate buffer) using an enzyme preparation (Sigma) with an activity of 52 units per g protein at 30 °C. Based on the recent isolation of a highly purified form of BPAO by Janes and Klinman (ca. 85% active),[17] the commercial enzyme we used must be ca. 11% pure.

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(19) Inactivation rates for 100 μM AMTMS at 5 °C in the absence and presence of 10 mM and 50 mM $PhCH_2NH_2$ were 0.34, 0.17, and 0.043 min^{-1}, respectively.

Despite the above observations, which are consistent with a mechanism-based inactivation,[20] *a spontaneous, temperature-dependent reactivation of BPAO occurred at later times* (see, e.g., Figure 1 plot without added salt).[21] This recovery is much faster than that observed in the AMTMS inactivation of MAO, for which an active site trimethylsilylation was initially considered, but which was ultimately shown through elegant kinetics and labeling experiments to involve enzyme attack on a $Me_3SiCH{=}NH_2^+$ species.[2] The rapid recovery seen with BPAO is not inconsistent with the expected hydrolytic lability of a trimethylsilylated heteroatom, which might be potentiated by F^-. In Figure 1, we show that 0.4 M KF alters the time scale of the interaction of AMTMS with BPAO at pH 9.0 no more than that seen with 0.4 M KCl.[22] Although this result fails to support an active-site trimethylsilylation, F^- could be ineffective on account of its tight hydration in aqueous medium and/or its inability to penetrate to the site of covalent modification.

Additional work will be required to clarify the nature of interaction of AMTMS with BPAO, including the use of α-deuterated AMTMS[2] to distinguish whether HCHO results from path A (eq 1) or path B with subsequent Brook rearrangement,[23] as well as studies using radiolabeled AMTMS and purified enzyme preparations. Nonetheless, the notion of an active-site-directed silylation, considered previously for MAO[2] and for inactivation of cytochrome $P\text{-}450_{scc}$ by a Me_3Si-containing steroid,[24] constitutes an intriguing protein modification tactic which may find important enzymologic applications using AMTMS-like agents.

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Supplementary Material Available: Experimental procedures and additional kinetic plots (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(20) Alternatively, there is a chance that AMTMS is acting as a "slow substrate" (k_i is then just k_{on}), the recovery of enzyme activity resulting from eventual turnover to noninhibiting products.

(21) The recovery of activity for enzyme inactivated with 100 μM AMTMS occurred with rates of 0.0099, 0.020, and 0.036 min^{-1} at 5, 10, and 30 °C.

(22) No alteration of the activity–time profile was observed using lower concentrations of KF or KCl and/or when the reactions were conducted at lower pH (7.2). Our observations using 0.4 M salts may represent an ionic strength effect.

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An Improved Procedure for the Synthesis and Use of $[RuCl_2(BINAP)]_2 \cdot NEt_3$. Dependence of the Ru(II)-BINAP Catalyzed Asymmetric Hydrogenation of β-Keto Esters on Trace Amounts of Acid

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Summary: The asymmetric hydrogenation of β-keto esters using 0.02–0.05 mol % Ru(II)–BINAP-derived catalyst can be conducted in a stardard Parr shaker apparatus at 40 °C and 30 psi of hydrogen in the presence of 0.1 mol % of a strong acid.

Asymmetric hydrogenation using the Ru(II)–BINAP system introduced by Noyori and co-workers provides very high enantioselectivity over a wide range of substrates with remarkable turnover.[1] However, all reports concerning the reduction of β-keto esters[2] suffer from the need for temperatures greater than 80 °C or hydrogen pressures greater than 1000 psi (80 atm) where special apparatus is required.[3] We communicate here that, in the presence of trace amounts of strong acid, asymmetric hydrogenation proceeds in a standard Parr shaker apparatus at low temperatures and readily attainable pressures (40 °C/30 psi H_2) with substrate/catalyst ratios up to 10 000. Additionally, we describe the kinetics of the reaction and a simple, reproducible procedure for preparation of purified catalyst.[3b,c]

The catalyst $[RuCl_2(BINAP)]_2 \cdot NEt_3$[4] is easily prepared using standard anaerobic benchtop techniques[3b,c] from commercially available (cyclooctadiene)ruthenium dichloride and (*R*)- or (*S*)-BINAP.[5] Filtration of the product using a double ended filter available from Kontes glass company provides a large measure of purification without the use of an inert atmosphere glovebox and avoids the need to remove large volumes of a high-boiling solvent in vacuo. We have used this technique to prepare from 200 mg–20 g of catalyst at a time. Reduction rates using this material are reproducible to ±10% across several batches. Although the catalyst must be stored under an inert atmosphere to prolong shelf life, it can be conveniently handled in air.

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