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Catalytic Hydrolysis of Esters of 2-Hydroxypyridine Derivatives for Cu^{2+} Detection

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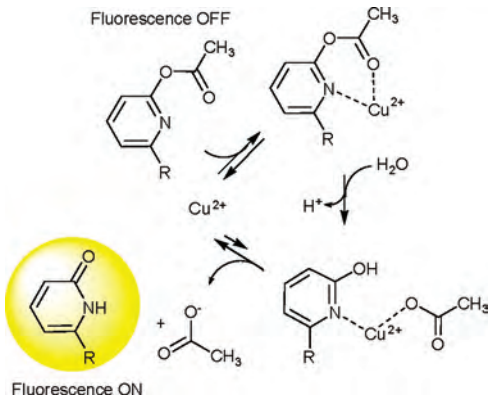
Hydrolysis of activated esters, e.g., picolinic acid and α-amino acid esters, in the presence of Cu^{2+} salts is a stoichiometric process because products of this reaction bind the catalytic metal ion substantially stronger than starting materials do. Herein we report improved ester substrates, which are cleaved in the presence of catalytic amounts of Cu^{2+}; 55 turnovers of hydrolysis are observed for the best substrate, acetic acid 2-hydroxypyridine ester. We demonstrate that this reaction can be used for sensitive and selective detection of Cu^{2+} by using both absorption and fluorescence spectroscopy.

Signal amplification is an efficient way of improving the sensitivity of analytical methods. Important examples include detection of nucleic acids by using a polymerase chain reaction (PCR), proteins by enzyme-linked immunosorbent assay (ELISA), and metals by methods based on the activation of enzymes.[1] Catalytic chemical reactions still do not find broad application in analysis because they are relatively slow and rarely reach high turnover (TN) numbers. Despite these disadvantages, ester cleavage,[2] S-alkylation[3] and native ligation[4] reactions were applied for the detection of single- and double-stranded DNAs. The Heck reaction[5] and dihydrofluorescein oxidation by H_2O_2[6] were used for Cu^{2+} detection.

Scheme 1. Catalytic Hydrolysis of 2-Hydroxypyridine Ester Substrates (**S**) in the Presence of Cu^{2+}



Ester groups that have a metal ion anchor in their proximity are quickly cleaved in aqueous solution at pH 7 in the presence of Cu^{2+}.[7] Typical substrates are esters of α-amino acids and picolinic acid. The corresponding hydrolysis products (acids) are stronger binders of Cu^{2+} than the esters; therefore, the hydrolysis does not go far beyond a single TN.[8] This limits application of the ester hydrolysis reaction in signal-amplified Cu^{2+} detection.

Herein we describe improved substrates for Cu^{2+}-catalyzed hydrolysis: esters of 2-hydroxypyridines,[9] **S**. A stable six-atom chelate is formed upon interaction of Cu^{2+} with **S**. Because the ester group is included in the chelate, its hydrolysis leads to the formation of products with lower denticity and, therefore, lower Cu^{2+} affinity. These products dissociate, releasing free Cu^{2+} that completes the catalytic cycle (Scheme 1). We demonstrate that hydrolysis of optimized substrates is catalytic with respect to Cu^{2+}, which can be used in Cu^{2+} analysis. Such esters should be defined as catalytic chemodosimeters because they accumulate and amplify the signal (dose) in response to the analyte and are irreversible.[10] Known chemodosimeters for Cu^{2+} detection

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(8) log K_a(Cu^{2+}-picolinic acid) = 8.6. (Suzuki, K.; et al. *J. Phys. Chem.* **1957**, *61*, 229). Stability of the Cu^{2+} complex with picolinic acid ester is unknown. The analogue of the ester is amide: log K_a(Cu^{2+}-picolinic acid amide) = 2.9. (Warnke, Z. *Rocz. Chem.* **1976**, *50*, 1801).

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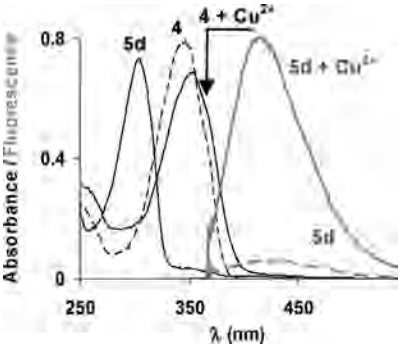
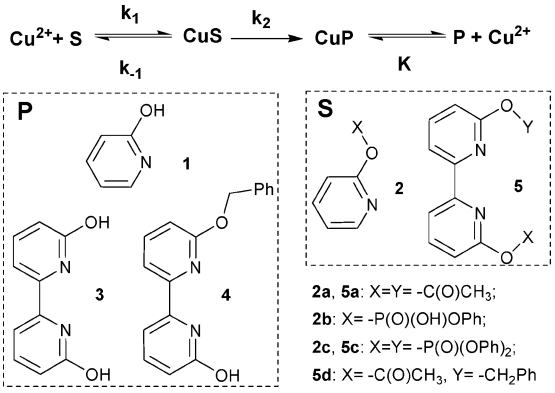


Figure 1. UV–visible absorption (black traces) and fluorescence (gray traces) spectra of substrate **5d** and its hydrolysis product **4** in the absence and presence of 1 equiv of Cu^{2+}. The fluorescence intensity is scaled down by a factor of 750. UV–vis spectra were acquired using [**5d**] = [**4**] = 50 μM; fluorescence spectra were acquired using [**5d**] = 1 μM and $\lambda_{ex} \sim 340$ nm. Buffer: MOPS (20 mM, pH* 7), NaCl (50 mM), DMSO/water (1/1, v/v). The fluorescence spectrum of a mixture of **5d** and Cu^{2+} was acquired 2 min after the addition of 1 equiv of Cu^{2+}. This spectrum coincides with the spectrum of **4** (1 μM), Cu^{2+} (1 μM) mixture.

Scheme 2. Studied Ester Substrates (**S**), Their Hydrolysis Products (**P**), and a Model of **S** Hydrolysis



reported by Yoon et al.,[11] Czarnik et al.,[12] Kierat and Kraemer,[13] and Mokhir et al.[14] are all based on stoichiometric metal-promoted reactions. Catalytic chemodosimers are potentially more sensitive.

Esters and products of their hydrolysis that were used in this study are summarized in Scheme 2. Their synthesis is described in the Supporting Information. Hydrolysis of the esters could be monitored by UV–visible spectroscopy because absorption maxima of the hydrolysis products are red-shifted with respect to those of their esters (Figure 1). Hydrolysis of **5a** and **5d** could also be studied by fluorescence spectroscopy because products of these reactions, **3** and **4**, exhibit intense violet fluorescence, whereas the substrates do not (Figure 1).

In the series of pyridine derivatives **2a**−**c**, acetic acid ester **2a** is most sensitive to Cu^{2+} (Table 1 and Figure 2). Other

Table 1. Kinetic Parameters of Hydrolysis of Some Studied Esters

	$10^2 k_2$[a] (min^{-1})	log K[b]	TN[c]
substrate (**S**)			
2a	7.6	4.5	44
2c	1.5	3.4	<1
5d	115	5.6	12
picolinic acid ethyl ester		~2.9[8]	~1
product (**P**)			
1		<2[d]	
3		7.4	
4		5.2	
picolinic acid		8.6[8]	
acetic acid		1.8[16]	

[a] k_2 = constant of pseudo first order. [b] K = formation constants of Cu^{2+}−**S** and Cu^{2+}−**P** complexes [c] TN = turnover number achieved 100 min after the beginning of the reaction. The signal-to-noise (S/N) ratio was ≥5. [d] **1** precipitates at [**1**] > 10 mM; therefore, accurate determination of the Cu−**1** stability was not possible.

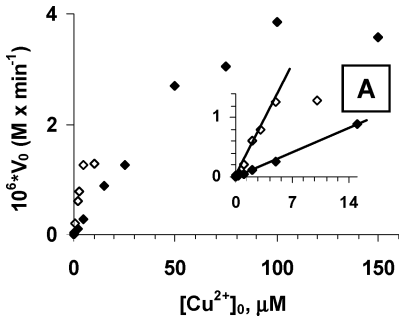


Figure 2. Dependence of the initial hydrolysis rate (V_0) of **2a** and **5d** on the total Cu^{2+} concentration ($[Cu^{2+}]_0$): [**2a**]$_0$ = 50 μM (filled diamonds); [**5d**]$_0$ = 1 μM (open diamonds). Buffer: MOPS (20 mM pH* 7), NaCl (50 mM), DMSO/water (1/1, v/v). T = 37 °C. Inset A: extension of the region of the plot at low $[Cu^{2+}]_0$; straight lines represent a linear fit of the data at low $[Cu^{2+}]_0$.

derivatives are either substantially less sensitive (phosphotriester **2c**) or not hydrolyzed at all even in the presence of 10 equiv of Cu^{2+} (phosphodiester **2b**). This may be explained by both the higher stability of the Cu−**2a** complex (log $K \sim 4.5$ vs 3.4 for Cu−**2c**) and its faster decomposition rate (k_2 = 0.076 vs 0.015 min^{-1} for Cu−**2c**).

At our experimental conditions ([**S**] = 50 μM), Cu^{2+} does not bind the products of **2a** hydrolysis: 2-hydroxypyridine (log K < 2)[15] and acetic acid (log K_1 = 1.8).[16] In contrast, the Cu^{2+} complex with the substrate **2a** is stable (log K = 4.5). As a consequence, one could expect efficient Cu^{2+}-catalyzed **2a** hydrolysis with low product inhibition. In practice, we observed 44 TNs of **2a** hydrolysis in 100 min (TN_{max} = 55). The reaction rate was constant over 10 TNs (Figure 3). At $[Cu^{2+}] < 1$ μM, substrate **2a** is a weak Cu^{2+} binder (Table 1). As a consequence, the rate of its catalytic hydrolysis is negligible at these conditions (Figure 2). To improve the sensitivity of the esters, we have prepared analogues of **2a** and **2c**: **5a**, **5c**, and **5d**. In these substrates, a pyridine residue was substituted for a 2,2′-bipyridine residue (Scheme 2). **5a**−**d** are potentially tri- and tetradentate Cu^{2+} binders and were expected to have high Cu^{2+} affinity. Moreover, products of **5a**−**d** hydrolysis, **3** and **4**, have high extinction coefficients and are fluorescent dyes exhibiting large Stokes shifts: ~80 nm (Figure 1).

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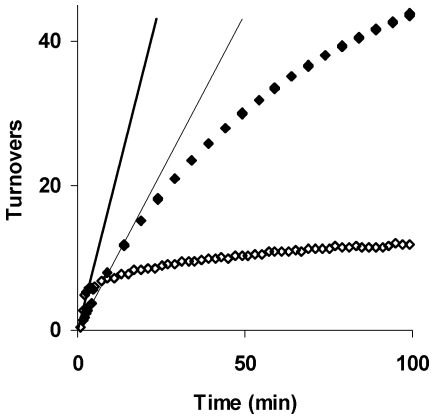

Figure 3. TNs of catalytic hydrolysis of **2a** and **5d**. Open diamonds: **5d** (100 μM), Cu^{2+} (300 nM). Filled diamonds: **2a** (1 mM), Cu^{2+} (3 μM). Buffer: see the caption to Figure 2. A linear fit of the data at the beginning of the reaction is shown as a straight line.

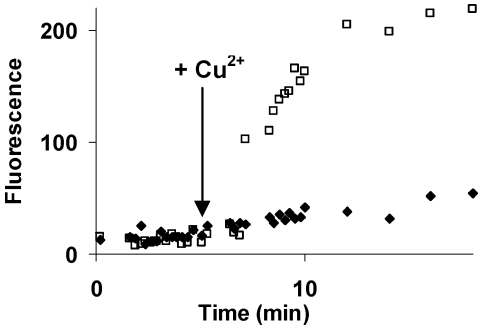

Figure 4. Selectivity of **5d** in Cu^{2+} detection. Fluorescence measurements: $\lambda_{ex} \sim 340$ nm, $\lambda_{em} \sim 420$ nm. Each mixture contains **5d** (100 nM), Zn^{2+}, Ni^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, and Ag^{+} (each 1 μM), Ca^{2+} and Mg^{2+} (each 10 μM), phosphate buffer (10 mM, pH 7), NaCl (50 mM), and DMSO/water (1/40, v/v). $T = 37$ °C. Filled diamonds: nothing else. Open squares: with Cu^{2+} (100 nM). The time point when Cu^{2+} was added is indicated with an arrow.

Within this series, the sensitivity of the substrates toward Cu^{2+} is decreased in the following order: **5d** > **5a** > **5c**. Surprisingly, tridentate **5d** is hydrolyzed 3.5 times faster in the presence of 1 equiv of Cu^{2+} than potentially tetradentate **5a**. This may indicate that **5a** ligates Cu^{2+} in a bidentate rather than a tetradentate fashion. The best substrate, **5d**, was selected for further investigations.

Cu^{2+} complexes with **5d** and its hydrolysis product, **4**, have similar stability (Table 1). Therefore, product inhibition is stronger in Cu^{2+}-catalyzed hydrolysis of **5d** than in that of **2a**. In particular, only ~12 TNs were reached after 100 min of **5d** (100 μM) hydrolysis in the presence of Cu^{2+} (300 nM). The hydrolysis rate was constant up to 5 TNs (Figure 3). Related 6,6′-bis(acylamino)-2,2′-bipyridines undergo Cu^{2+}-catalyzed decomposition in mixtures of methanol and water. In this reaction, ~2 TNs were reached within ~500 min.[17] In contrast, stoichiometric amounts of Cu^{2+} are required for hydrolysis of carbamate of a 2-hydroxypyridine derivative reported by Zhau and co-workers. Moreover, hydrolysis of this substrate requires very high Cu^{2+} concentration, 1 mM.[18] As we mentioned before, reactions of Cu^{2+}-catalyzed hydrolysis of picolinic acid and α-amino acid esters are also stoichiometric. Thus, reactions of **2a** and **5d** hydrolysis exhibit the highest TN numbers observed in known, up to date chemical hydrolytic reactions.

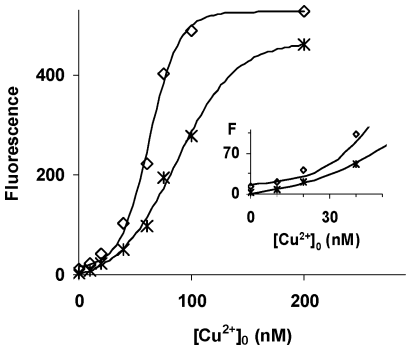

Figure 5. Change of the fluorescence intensity ($\lambda_{ex} \sim 340$ nm and $\lambda_{em} \sim 420$ nm) of a **5d** (5 μM) solution in response to different concentrations of Cu^{2+}. Fluorescence was measured 2.6 (crosses) and 19 min (open diamonds) after the addition of Cu^{2+}. Buffer: see the caption to Figure 3. Inset: expanded region of the plot in the low $[Cu^{2+}]_0$ range.

We have done the experiments that have been discussed up to now in dimethyl sulfoxide (DMSO)/water (1/1, v/v) solutions. At these conditions, all prepared substrates and their Cu^{2+} complexes are soluble and their direct comparison is possible. The best found substrates **2a** and **5d** are well soluble in water at pH 7. Therefore, we have used them for Cu^{2+} analysis at more realistic conditions. In aqueous buffers, both substrates are quite Cu^{2+}-specific. In particular, they are not hydrolyzed by 10-fold excess Fe^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Co^{2+}, Mn^{2+}, and Hg^{2+} and 100-fold excess Mg^{2+} and Ca^{2+}. **5d** can be used for analysis of Cu^{2+} directly in physiological buffers because catalytic hydrolysis of **5d** seems to not be inhibited by phosphates (Figure 4).

Only 2.6 min after the addition of 40−200 nM Cu^{2+}, fluorescence of **5d** is increased by >50 fold. When 10 nM < $[Cu^{2+}]_0$ < 40 nM solutions are analyzed, the waiting time should be extended to 19 min (inset, Figure 5). Ten nmolar Cu^{2+} is the lowest concentration that we could detect using **5d**. In this case, the waiting time should be extended to 80 min to achieve a signal-to-noise ratio ~ 5.[15]

In summary, we have prepared ester substrates that can be cleaved in aqueous solution at pH 7 in the presence of catalytic amounts of Cu^{2+}. A total of 55 catalytic TNs were achieved with substrate **2a** and 12 TNs with substrate **5d**. These TN numbers are the largest ones observed in hydrolytic chemical reactions. We have demonstrated that our reaction can be used for analysis of Cu^{2+}.

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Supporting Information Available: The synthesis of new substrates is described. This material is available free of charge via the Internet at http://pubs.acs.org.

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