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Silica-bound copper(II) triazacyclononane: a robust material for the heterogeneous hydrolysis of a phosphodiester

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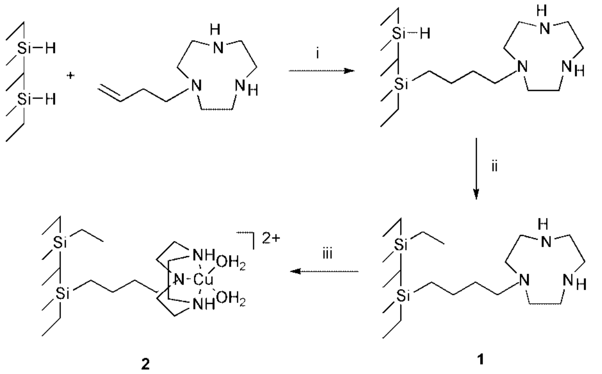
Copper(II) triazacyclononane, a catalyst for the hydrolysis of phosphate esters, has been covalently immobilized onto a silica surface and the resulting compound is a robust material that can effect heterogeneous phosphodiester hydrolysis.

The exceptional stability of phosphodiesters makes their cleavage a challenging task: at neutral pH and 25 °C, the half-life for the hydrolysis of dimethyl phosphate is estimated to be 130 000 years.[1] It is well documented, however, that a large range of metal ions and metal complexes are able to dramatically increase the rate of hydrolysis of phosphodiesters in aqueous solutions.[2] We have shown that in aqueous solution dichloro(1,4,7-triazacyclononane)copper(II) ($Cu[9]aneN_3Cl_2$) catalytically hydrolyzes the activated phosphodiester, bis(4-nitrophenyl)phosphate (BNPP).[3] A drawback of these homogeneous catalysis reactions is the inability to recover and reuse the catalyst. A heterogeneous catalyst, however, formed by immobilization of the active complex onto a solid support, can be easily separated from the reaction products and recycled continuously. Here we report the synthesis and characterization of silica-bound $(Cu[9]aneN_3)^{2+}$ and its reaction chemistry with BNPP.

Silica-bound $(Cu[9]aneN_3)^{2+}$ was prepared by a rhodium-catalyzed hydrosilation reaction between *N*-(4-but-1-enyl)-1,4,7-triazacyclononane[4] and hydride-modified silica,[5] both of which were prepared according to literature procedures. The chosen method of immobilization was favored because it produced more uniform and hydrolytically stable silicon–alkyl group linkages.[6] Conventional methods usually involve immobilization through an amide bond, which is not appropriate because $(Cu[9]aneN_3)^{2+}$ has been shown to hydrolyze amides.[7] *N*-(4-but-1-enyl)-1,4,7-triazacyclononane and hydride-modified silica were refluxed in toluene in the presence of Wilkinson's catalyst $[RhCl(PPh_3)_3]$ for 3 days, covalently linking the macrocycle to the silica surface (Scheme 1).[8] Unreacted hydrides were blocked with ethyl groups by charging the flask with ethylene (1 atm) and vigorously stirring the reaction solution for 15 h at room temperature.[9] After thorough washes with toluene, dioxane, and acetone, silica-bound triazacyclononane **1**, a pale yellow solid, was collected and dried at room temperature.



Scheme 1 *Reagents and conditions*: i, $RhCl(PPh_3)_3$, toluene, reflux, 3 days; ii, C_2H_4, $RhCl(PPh_3)_3$, toluene, 25 °C, 15 h; iii, $Cu(NO_3)_2$ (aq).

Characterization of **1** reveals that the silica surface has been modified with an organic layer. Elemental analysis was performed on **1**, yielding the following mass percentages: C 5.14%, H 1.54% and N 1.11%.[10] Based on the nitrogen value, it was calculated that the surface concentration of triazacyclononane was 0.264 mmol (g silica)$^{-1}$. Additionally, it was estimated that, of the original hydrides present on the silica surface, 24% had reacted with the *N*-(4-but-1-enyl)-1,4,7-triazacyclononane. Both of these values agree well with other reported values for silica-bound macrocycles.[11]

Metallation of **1** was achieved by mixing an aqueous solution of copper(II) nitrate with **1** and stirring vigorously for 30 min. The yellow material quickly became blue. The solid was isolated by filtration over a glass frit and was repeatedly washed with water until the washings were colorless. Finally, the material was washed twice with methanol and allowed to air dry, resulting in a pale blue solid, silica-bound copper(II) triazacyclononane **2**. EPR spectroscopy was used to probe the copper(II) environments in **2**. Spectra were recorded at both room temperature and 77 K.[12] The 77 K spectrum of **2** showed the characteristics of an axially symmetric d^9 copper complex.[13] The room temperature spectrum of **2** (Fig. 1) was essentially identical to its 77 K spectrum. A broadened spectrum was not observed, revealing that spin–spin interactions between the copper centers are minor or non-existent. Therefore, the conclusion can be drawn that the copper complexes anchored to the silica surface are well separated and non-interacting. This result demonstrates that dimerization of $(Cu[9]aneN_3)^{2+}$, an occurrence that contributes to rate reduction in the hydrolysis of phosphodiesters in aqueous solution,[3] will not be a factor for reactions with **2**.

Kinetic experiments showed that **2** was effective at hydrolyzing BNPP. Typical experiments began with the addition of 60 mg **2** to 50 mM HEPES [*N*-(2-hydroxyethyl)piperazine-*N′*-ethanesulfonic acid] buffer at pH 7.8. The solution was stirred

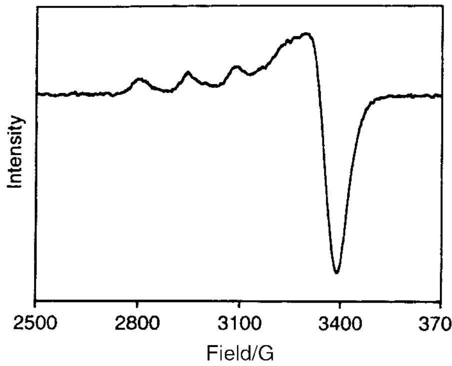


Fig. 1 X-Band EPR spectrum of **2** (undiluted powder sample). The spectrum was recorded at 298 K, 101 mW microwave power, 3.2 × 10^5 receiver gain, 5.88 G modulation amplitude, 9.6469 GHz microwave frequency, 100 kHz modulation frequency and a 163.84 ms time constant, using 10 averaged scans, each containing 4096 data points; $g_\perp$ = 2.065, $g_\parallel$ = 2.283, A = 143 G.

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vigorously for 15 min to wet the solid, followed by addition of 5 mM BNPP, to a total reaction volume of 3 mL. The solution was allowed to stir in the dark at 25 °C and was monitored by visible spectrophotometry, following the absorption at 400 nm due to the hydrolysis product 4-nitrophenolate.[14] Fig. 2(a) shows a plot of concentration of 4-nitrophenolate *vs.* time for a typical reaction. The plot shows an extended induction period where no hydrolysis was observed, and then a linear increase in product formation followed by a second linear region of product formation at a slower rate. The slowing of the reaction at relatively low (20%) conversion suggests that product inhibition may be significant. When **2** was allowed to stir with the buffer for 5 days prior to the addition of substrate, a significantly reduced induction period was observed, indicating that solvation of the solid surface is a limitation in this system. Negligible BNPP hydrolysis was observed in the absence of **2** or in the presence of **1** at the same temperature and pH over a two week period.

The same portion of **2** can be reused to hydrolyze additional samples of BNPP. In an initial reaction cycle, 60 mg **2** was combined with 5 mM BNPP in 50 mM HEPES, pH 7.8, as above. After *ca.* 700 h, by which time the reaction was proceeding at the slower rate, **2** was isolated from the suspension and repeatedly washed with 1 mL aliquots of 50 mM HEPES buffer (pH 7.8) until the washings were colorless (at least five washings). Fresh HEPES and BNPP (identical concentrations as in cycle 1) were added to **2** and the 4-nitrophenolate concentration was monitored as a function of time. In the second cycle, hydrolysis was again observed, following the same reaction profile as the first cycle with the exception of a shorter induction period (data not shown). This same portion of **2** was recycled a third time following the same procedure as for the second cycle, and identical hydrolytic activity was observed.

When **2** was recycled while the hydrolysis rate was maximal, after *ca.* 150 h, the higher reaction rate could be maintained through repeated reaction cycles. In the first reaction cycle 60 mg **2** and 5 mM BNPP were reacted at 25 °C, pH 7.8 (50 mM HEPES) for 300 h, and slow hydrolysis was observed. After isolation and washing by the procedure described above, **2** was combined with fresh substrate and buffer under the same conditions as in the first cycle. The reaction was followed for *ca.* 150 h. The rate of hydrolysis in the second cycle was significantly greater than in the first. The recycling procedure was repeated through 12 cycles over the course of 3 months: Fig. 2(b) shows data for cycles 1, 4 and 7. Cycles 1–5 proceeded with progressively increasing reaction rates. Once the maximal rate was achieved at cycle 5, all subsequent cycles exhibited the same rate of reaction. The maximal observed rate of hydrolysis was 7.5×10^{-10} M s^{-1}; this rate is significantly faster than the uncatalyzed rate, which cannot be measured at room temperature and neutral pH. These data reveal that if **2** is isolated, washed and recycled while reacting at its maximal rate, the high rate can be maintained through repeated cycles for an extended period of time.

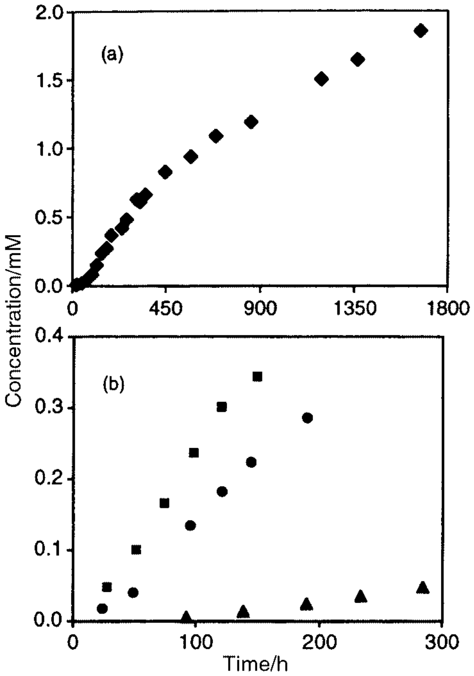

Fig. 2 (a) Single reaction time course. Plot of concentration of 4-nitrophenolate *vs.* time for **2**-mediated hydrolysis of BNPP. Reactions were carried out at pH 7.8, maintained by 50 mM HEPES, and 25 °C. The substrate (5 mM BNPP) was added to 60 mg **2**, and a suspension was maintained by constant stirring. Aliquots were clarified by centrifugation and filtration for absorbance measurements. (b) Multiple cycle, initial rate time course. Plot of concentration of 4-nitrophenolate *vs.* time for **2**-mediated hydrolysis of BNPP: see (a) for conditions. Each reaction after cycle 1 was allowed to proceed to *ca.* 6% conversion to product before recycling; (▲) cycle 1, (●) cycle 4, (■) cycle 7.

The data presented herein demonstrate effective heterogeneous hydrolysis of a phosphodiester promoted by a surface-immobilized copper(II) macrocycle, (Cu[9]aneN$_3$)$^{2+}$. Importantly, **2** can be reused over many months to hydrolyze additional portions of BNPP, and a higher reaction rate can be maintained at low percentage conversion to product. The hydrophobic nature of the material is likely responsible for the long induction period and work is currently underway to promote the silica–substrate interaction.

Notes and references

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13 The X-band EPR spectrum of **2** (undiluted powder sample) was recorded at 77 K, 15.98 mW microwave power, 1.6×10^3 receiver gain, 18.593 G modulation amplitude, 9.2215 GHz microwave frequency, 100 kHz modulation frequency, and a 163.84 ms time constant, using 10 averaged scans, each containing 4096 data points: $g_{\perp} = 2.064$, $g_{\parallel} = 2.285$, $A = 148$ G.

14 Aqueous solutions were prepared with water purified by passage through a Millipore purification system. Kinetic measurements were made in 1.00 cm pathlength quartz cells with the use of a Hitachi U-3210 UV/visible spectrophotometer. Correction for the spontaneous hydrolysis of BNPP was accomplished by direct difference to a reference cell identical in all respects except lacking **2**. The concentration of 4-nitrophenolate produced was calculated from the extinction coefficient (18 700 M^{-1} cm^{-1}). Concentrations were corrected for the degree of ionization of the 4-nitrophenol at the pH and reaction temperature; see: A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum Press, New York, 1977.