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Poly(ethylene glycol)-supported copper(II) triazacyclononane: an efficient, recoverable, and recyclable catalyst for the cleavage of a phosphodiester

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Abstract—1,3,5-Triazacyclononane macrocycle has been linked to the soluble polymer monomethoxy poly(ethyleneglycol) and its copper(II) complex efficiently catalyzes the hydrolysis of a model phosphodiester. The catalyst, easily recovered from the water solution, shows no appreciable loss of activity when recycled up to three times. © 2002 Elsevier Science Ltd. All rights reserved.

The hydrolytic cleavage of phosphoric esters is an important reaction both in environmental and in biological chemistry.[1] However, these substrates and in particular phosphodiesters are exceptionally stable toward hydrolysis: at neutral pH and 25°C the half-life for the cleavage of dimethyl phosphate is estimated to be 130 000 years.[2] As a consequence, much effort has been devoted to the development of efficient catalysts for this reaction. It is well documented that a variety of metal ions and metal complexes are able to dramatically increase the rate of hydrolysis of phosphodiesters in water solution.[3] A drawback of these homogeneous catalyses is the impossibility to recover and recycle the catalyst. Immobilization of the catalysts on an insoluble matrix can provide a simple solution to this problem.[4] For example, copper(II) complexes have been incorporated into insoluble polymers[5] or linked to silica particles,[6] obtaining heterogeneous catalysts active in the cleavage of phosphoric esters. In this context, the ideal polymer support should allow the complete solubility of the catalysts in some solvents, in order to maintain the advantages of homogeneous catalysis, and insolubility in other solvents, so that the supported catalyst can easily be isolated and recovered by precipitation and filtration.[7] Modified poly(ethylene glycol)s (PEGs) of Mw>2000 Da are readily functionalized polymers that feature these convenient solubility properties.[8] Recently, the mono methyl ether of PEG_{5000} (MPEG) has successfully been used for the immobilization of different reactive systems[9] used, for example, as phase transfer catalysts[7] and in hydrogenation,[10] epoxidation,[11] and carbon–carbon bond formation.[12] To the best of our knowledge, however, a copper(II) complex has never been attached to PEG and used as a recoverable catalyst for the hydrolytic cleavage of a phosphodiester.

For this reason, we argued that a triazacyclononane (TACN)-functionalized MPEG would be a particularly interesting target because: (i) the reported complexation constant for the binding of copper(II) by this macrocycle is very high ($\log K = 15.5$)[13]; (ii) extensive work by Burstyn and co-workers has highlighted the efficiency of the copper(II) complex of this macrocycle in the hydrolysis of phosphate diesters[14] and carboxamides[15] as well; and (iii) the conjugation of the catalyst to the MPEG polymer would allow an easy recovery and reuse of the catalyst.

The preparation of the MPEG-linked catalyst is shown in Scheme 1. 1,6-Aminohexanol was CBz-protected at the amino nitrogen and the hydroxyl group was converted into the corresponding bromide **3**. Reaction of the bromide with the diprotected triazacyclonane,[16] followed by removal of the CBz group, led to compound

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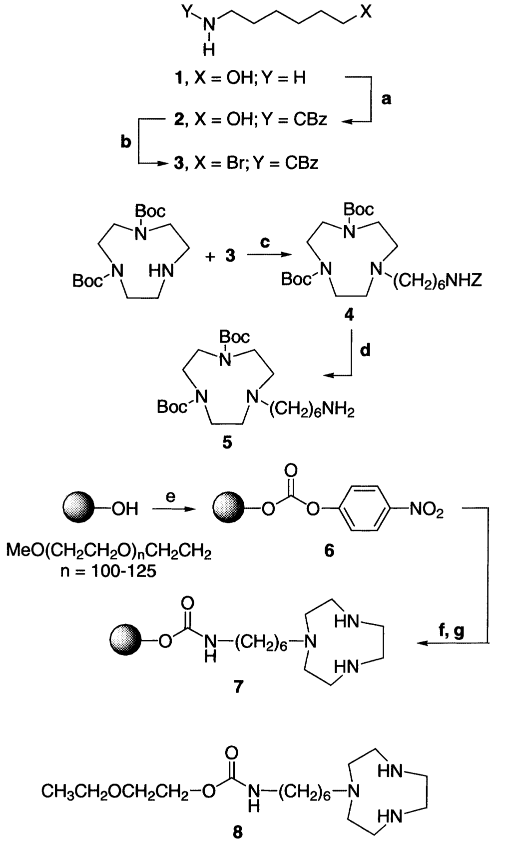
5 ready to be connected to the MPEG polymer. On the basis of our experience[17] we decided to use a carbamate linkage for this connection. The terminal hydroxyl group of MPEG (Mw = 5000 Da) was reacted with 4-nitrophenyl chloroformate obtaining the 4-nitrophenyl carbonate **6**. Nucleophilic displacement of the 4-nitrophenyl group with **5** followed by deprotection of the amines led to the target compound **7**. For comparison purposes and using the same synthetic scheme we prepared compound **8** in which the PEG polymer has been substituted with a shorter ethoxyethyl ether.[18]



Scheme 1. (a) CBzCl, MeOH, Et$_3$N, rt, 5 h; (b) CBr$_4$, Ph$_3$P, CH$_2$Cl$_2$, rt 1.5 h; (c) DIPEA, CH$_3$CN, reflux 10 h; (d) H$_2$, Pd/C, MeOH, rt, 5 h; (e) 4-NO$_2$PhOCOCl, TEA, CH$_2$Cl$_2$, rt, overnight; (f) **5**, CH$_2$Cl$_2$, rt, 48 h; (g) TFA/CH$_2$Cl$_2$ 1:1, rt, 4 h.

In water at pH 7.8 the MPEG-conjugate triazacyclononane binds copper(II) as shown by the appearance of an absorption band at 620 nm in the UV–vis spectrum. Figure 1 shows the UV–vis titration of **7** and TACN with copper(II): in both cases the absorbance increases linearly up to 1 equiv. of metal ion and then levels off though to a different final value. This is a clear indication of a strong binding with a 1:1 stoichiometry and that the conjugation to the polymer does not alter substantially the complexation properties of the azamacrocycle but for a slight increase in the molar extinction coefficient of the complex. Moreover, by using compound **8** we checked the stability of the carbamate linkage under the hydrolytic conditions used. After 1 week at acidic (pH 2), basic (pH 10) and almost neutral pH (8), in the presence or absence of copper(II), no evidence of hydrolytic degradation was observed using ^{1}H NMR after removal of the metal ion.

Kinetic studies were performed by using bis(4-nitrophenyl)phosphate (BNPP) which is considered a reference substrate for the cleavage of phosphodiesters.[3] Figure 2 reports the kinetic profiles for the cleavage of BNPP (0.1 mM) at pH 7.8 and 40°C in the presence of increasing concentrations of the copper(II) complexes of compounds **7**, **8**, and TACN.[19]

Analysis of the Fig. 2 shows that the order of reactivity is **7**>**8**>TACN: at 0.8 mM concentration **7** is about 25% more reactive than **8** and more than threefold than TACN. The maximum apparent rate constant observed for **7** is 4.5×10^{-6} s^{-1} which is considerably faster than the uncatalyzed rate of cleavage of BNPP.[20] However, the three kinetic profiles are not linear and show a downward curvature. This behavior has been already observed for TACN[14] and for other polyamine ligands[21] and has been attributed to a monomer–dimer equilibrium where the monomer is a more active catalyst. Increasing the concentration of copper(II) complex the dimer, probably a bis(μ-hydroxide)-bridged species, is increasingly formed and the reaction rate decreases.

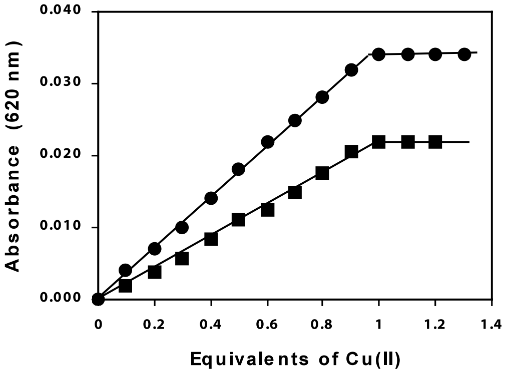


Figure 1. UV–vis titration of **7** (●) and TACN (■) with Cu(II). Conditions: [ligand] = 5×10^{-4} M, HEPES buffer 50 mM, pH 7.8, 25°C.

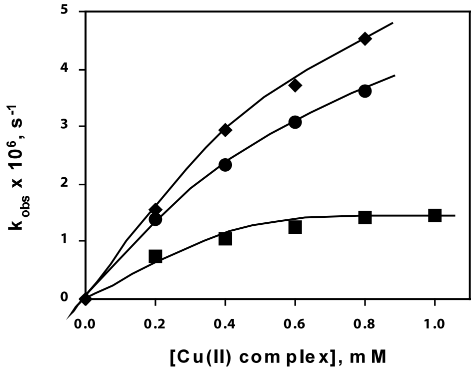


Figure 2. Kinetic profiles for the cleavage of BNPP (0.1 mM) in the presence of the copper(II) complexes of **7** (◆), **8** (●), and TACN (■) at 40°C and pH 7.8 (HEPES buffer, 50 mM).

By fitting the data[14] it is possible to obtain the apparent dimerization constants which are 840, 1060, and 2800 M^{-1} for **7**, **8**, and TACN, respectively. Clearly, substitution at one macrocycle nitrogen reduces the dimerization constant[22] and this is reflected in a higher reactivity.

To verify if the MPEG-conjugate copper(II) complex is a real catalyst we investigated its kinetic behavior under conditions of excess substrate. Figure 3 shows a typical kinetic experiment where BNPP is five times more concentrated than the copper(II) complex of ligand **7**.

The concentration of the hydrolyzed product increases smoothly with time following first order kinetics. After about 160 h of reaction more than 75% of the initial BNPP is hydrolyzed in 3.7 turn-over cycles of the catalyst. Moreover, the regular profile of Figure 3 shows that product inhibition is not significant, at least under these experimental conditions.

Finally, we investigated the possibility to recover and reuse the MPEG-conjugate catalyst. Different conditions for the extraction of the polymer from the reaction medium were tested: acidic (pH 1), basic (pH 12) in the presence of EDTA, and direct extraction from the water solution buffered at pH 7.8. This last procedure was found to be the most convenient also because the copper(II) complex of **7**, i.e. the whole catalytic system, is directly obtained ready to be reused. However, the direct extraction of the water phase with methylene chloride was not satisfactory especially when using small amount of MPEG-conjugate. A higher recovery yield was obtained by lyophilization of the water solution and extraction of the solid residue with methylene chloride followed by precipitation of the MPEG derivative with ethyl ether. Using this procedure more than 80% of the copper(II) complex of **7** was recovered from the reaction medium in the conditions of the kinetic experiments, where the maximum amount of **7** used was limited to 10–15 mg to avoid excessive absorbance of the samples. However, the recovery yield increases substantially (up to 97%) when the reactions are performed on larger scale, employing larger amounts of PEG-conjugate.

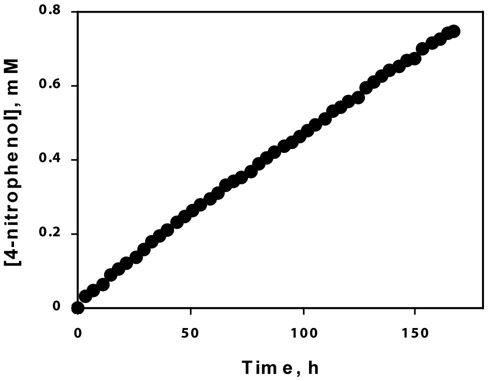


Figure 3. Plot of concentration of 4-nitrophenol versus time for the hydrolysis of BNPP (1.0 mM) catalyzed by **7** (0.2 mM) at pH 7.8 (HEPES buffer, 50 mM) and 40°C. The reaction was followed at 450 nm in order to keep the final absorbance below 2.

The hydrolytic efficiency of the recovered catalyst was tested in the following experiment: 11 mg of **7** was dissolved in HEPES buffer (50 mM, pH 7.8) and 1 equiv. of copper(II) was added to form the metal ion complex. The solution was placed in a cuvette and the substrate was added. The final volume and concentrations were: 1.0 mL, [**7**·Cu(II)] = 2.1 mM, [BNPP] = 2.0 mM. The reaction was followed at 40°C, monitoring the increase in absorbance at 440 nm for 12 h until about 10% of the substrate was hydrolyzed. The copper(II) complex of **7** was then recovered from the water solution as described above. The recovered catalyst was dissolved in fresh buffer and its concentration was determined using the typical absorbance band of the metal ion complex at 620 nm. A new aliquot of BNPP was then added in order to obtain an identical final volume and substrate concentration as in cycle 1. The reaction was monitored for about 12 h and then the catalyst was again recovered and reused in a third cycle. The kinetic data relative to this experiment are collected in Table 1.

As said above, the use of a relatively small amount of MPEG-conjugate hampers the full recovery of the catalyst. In the recycling experiments the yield of recovery was around 80% and, the total volume of the water solution being the same, the concentration of catalyst decreases in the subsequent cycles. As a consequence, the apparent pseudo-first order rate constant (k'_{obs}) of the reaction decreases on going from the first to the last cycle. However, if the decrease of concentration is taken into account and the comparison is made using the apparent second order rate constants ($k''_{obs} = k'_{obs}/$[**7**·Cu(II)]), it is evident that the intrinsic reactivity of the MPEG-conjugate is the same in the three cycles and no activity has been lost.

In conclusion, the immobilization of the TACN macrocycle on a modified MPEG provides an efficient catalyst for the copper(II) promoted cleavage of a model

Table 1. Kinetic parameters for the cleavage of BNPP by **7**·Cu(II) in three subsequent reaction cycles[a]

Cycle	% of recovering	[**7**·Cu(II)] (mM)	$k'_{obs} \times 10^6$ (s^{-1})	$k''_{obs} \times 10^3$ (s^{-1} M^{-1})
1	–	2.1	9.6	4.6
2	85	1.7	8.6	5.0
3	81	1.4	6.0	4.3

[a] [BNPP] = 2.0 mM, HEPES buffer 50 mM, pH 7.8, 40°C.

phosphate diester. The catalyst can be easily recovered and reused without appreciable loss of activity. Moreover, the system presents all the advantages of an homogeneous catalyst without the problems often connected to a heterogeneous one. For instance, when compared to an insoluble catalyst obtained by immobilization of TACN on silica[6] our system is about 40-fold more reactive without the need of a high stirring rate or large conditioning time to ensure accessibility of the substrate to the catalytic site. These results open the way to a new class of supported hydrolytic catalysts and work is in progress to obtain more efficient systems active toward phosphate esters and other relevant substrates.

Acknowledgements

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18. Selected analytical data for **7**: ^{1}H NMR δ 5.15 (br, 1H, N*H*), 4.19 (m, 2H, -CH_2-OCONH-), 3.89–3.40 (m, 454H PEG, 4H -CH_2(5,6)-NH-), 3.34 (s, 3H, CH_3O-), 3.15 (m, 6H, -CH_2(3,8)-NH-, -CH$_2$-CH_2(6)-NHCOO-), 2.87 (m, 4H, -CH_2(2,9)-N-), 2.63 (brt, 2H, -CH_2(1)-N-TACN), 1.47 (m, 4H, -CH_2(5,2)), 1.30 (m, 4H, -CH_2(3,4)). ESI MS: (1:1 MeOH/5% CH_3COONa) mass increments between native and functionalized PEG **7** calculated (63.5 Th) and measured (63.2 Th) for the four charge cluster are in accordance.
19. Reactions were monitored by following the absorbance at 400 nm of the hydrolysis product 4-nitrophenolate. The pseudo first order rate constants were obtained using the method of initial rates. The absorbance versus time data of the first linear part of the reaction (up to 10% conversion) were converted in concentration versus time data using the extinction coefficient for 4-nitrophenolate ($\varepsilon = 18\,700$ M^{-1} cm^{-1}). Concentrations were corrected for the degree of ionization of the 4-nitrophenol at the operative pH. The rate constants were obtained by dividing the slope of this initial linear part of the kinetic by the analytical concentration of BNPP.
20. On the basis of the available literature date, the rate of the spontaneous hydrolysis of BNPP in the conditions employed in the kinetic experiments can be estimated to be about 5×10^{-11} s^{-1}. See: Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, *113*, 8935.
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