Phosphate diesters cleavage mediated by Ce(IV) complexes self-assembled on gold nanoparticles†

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Received 23rd December 2009, Accepted 8th March 2010
First published as an Advance Article on the web 29th March 2010
DOI: 10.1039/b926916a

Monolayer protected gold nanoparticles (MPGN) coated with metal chelating groups were prepared. In the presence of Ce(IV), the MPGN proved to be highly active in promoting the hydrolytic cleavage of the DNA model bis-*p*-nitrophenyl phosphate (BNP). A 2.5 million-fold rate acceleration of the BNP hydrolysis was measured at a concentration of Ce(IV) as low as 120 μM (pH 7, 25 °C). This corresponds to a reduction of the substrate half-life time from 2000 years to few hours. The MPGN-Ce(IV) system is 1 to 2 orders of magnitude more reactive than any mono- and binuclear Ce(IV) complex so far reported. The source of such reactivity is the cooperation between the metal ions self-organized on the surface of the nanoparticles.

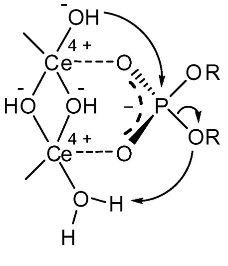
Introduction

Phosphate diester bonds are present in many relevant biomolecules, such as nucleic and ribonucleic acids. These bonds are very resistant to hydrolysis. In fact, when the sole hydrolytic cleavage of the P–O bond is taken in consideration, the half-life time of phosphodiester groups in DNA under physiological conditions (pH 7, 25 °C) has been estimated to be 200 million years.[1] Even in the case of an activated derivative like bis-*p*-nitrophenyl phosphate (BNP), often used as a DNA model, the half-life time is as long as 2000 years under the same conditions.[2] On the other hand, the enzymes that hydrolyze phosphodiester bonds in living organisms, such as nucleases and topoisomerases, are able to cleave them in a few milliseconds, reaching rate accelerations up to 10^{18}.[3] This makes the discovery of synthetic hydrolytic agents capable to promote the cleavage of phosphate diesters a stimulating intellectual challenge. Moreover, artificial hydrolytic agents could also find several useful applications in the medical, environmental and biological fields, as restriction agents, pesticides detoxificants, *anti*-DNA or *anti*-RNA drugs.[4]

Even if the reactivity of artificial agents is still far from that of enzymes, several hydrolytic catalysts have been reported, usually based on Cu(II), Fe(III), Zn(II), Co(III) and Ce(IV) complexes, the last being in many cases the most active.[4a]

Among lanthanides, cerium is peculiar for its ability to reach a stable tetravalent oxidation state besides the usual trivalent one. Still it cleaves phosphate diesters and nucleic acids without oxidative mechanisms.[5] In addition, Ce(IV) features a high coordination number (up to 12) and a fast ligand exchange rate. These features, along with its great electron-withdrawing ability due to its high oxidation state, make the Ce(IV) ion extremely efficient in hydrolyzing phosphate diesters.[6] At pH 7 and 50 °C, Ce(IV) reduces the half-life of DNA to a few hours and the rate of the reaction is constant for pH values ranging from 2 to 8.5.[5]

On the other hand, the use of Ce(IV) ions present some disadvantages. The most important one is that in aqueous solutions Ce(IV) forms hydroxopolymers and many different hydroxocomplexes below pH 4, and precipitate as Ce(IV) hydroxide above pH 4.[5,7] Such cerium clusters and gels are highly active in the cleavage of dinucleotides, single strand DNA, and double strand (but not plasmid)[8] DNA,[5] but the reproducibility is clearly poor, since the slow aggregation and precipitation of the hydroxide from the solutions reduces their activity depending on the aging time.[7] In any case, all the active species so far postulated for Ce(IV) aqueous solutions are multinuclear hydroxocomplexes, such as $Ce_2(OH)_4^{4+}$, $Ce_2(OH)_7^{+}$, and its dimer $Ce_4(OH)_{15}^{+}$.[7] The mechanism proposed (Scheme 1) involves the coordination of the phosphate to two Ce(IV) ions through the oxygen atoms. This strongly activates the phosphorus toward the nucleophile attack. Beside substrate activation, the other roles of the two metal ions are the formation of the nucleophile (a metal-bound hydroxide), the stabilization of the negatively-charged transition state, and the leaving group activation though hydrogen transfer from a metal bound water molecule.[5]



Scheme 1 Proposed mechanism for phosphate diester cleavage promoted by Ce(IV) polymetallic clusters.

To obtain more stable systems, Ce(IV) may be employed as a complex. Many ligands with different structures were tested: polyamines, polyalcohols, ethers and carboxylates.[8,9] Unfortunately, the reactivity of such complexes is often lower than that

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† Electronic supplementary information (ESI) available: Experimental procedures, synthesis and characterization of **4**, **5**, and of the MPGNs, additional kinetic experiments. See DOI: 10.1039/b926916a

of the free ion. In fact, complexation prevents the formation of polynuclear active species, reduces the number of coordination sites available on the metal and partially neutralizes the charge of the ion. However, in some cases peculiar and interesting reactivities have been observed.

In particular, Ce(IV)/EDTA hydrolyzes single strand DNA selectively over double strand DNA[10] and this made possible the development of the ARCUT system proposed by Komiyama and co-workers, which is the only artificial restriction agent known to date.[11]

Higher reactivity is obtained with bimetallic complexes, that partially reproduce the polymetallic arrangement of Ce(IV) gels. In particular, the Ce_2(HXTA) complex (Chart 1) cleaves plasmid DNA with a rate constant of 1.4×10^{-4} s^{-1} (pH 8, 37 °C), which corresponds to an half-life time of 1.4 h for the nicking process, at a concentration as low as 10 μM.[12] Interestingly, double strand scission is preferred. The mechanism proposed for the reaction is similar to the one reported for the ligandless system (Scheme 1).

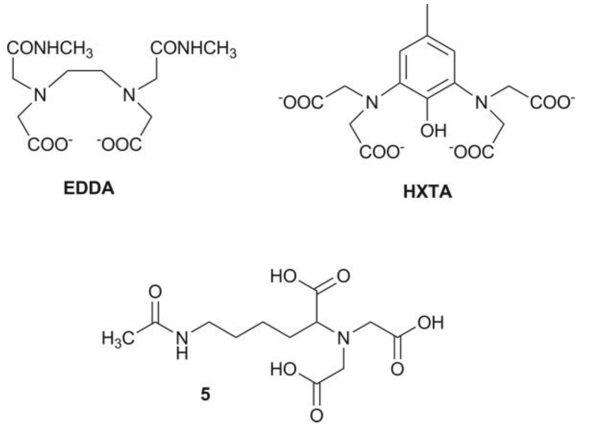


Chart 1 Ligands used to obtain stable Ce(IV) complexes.

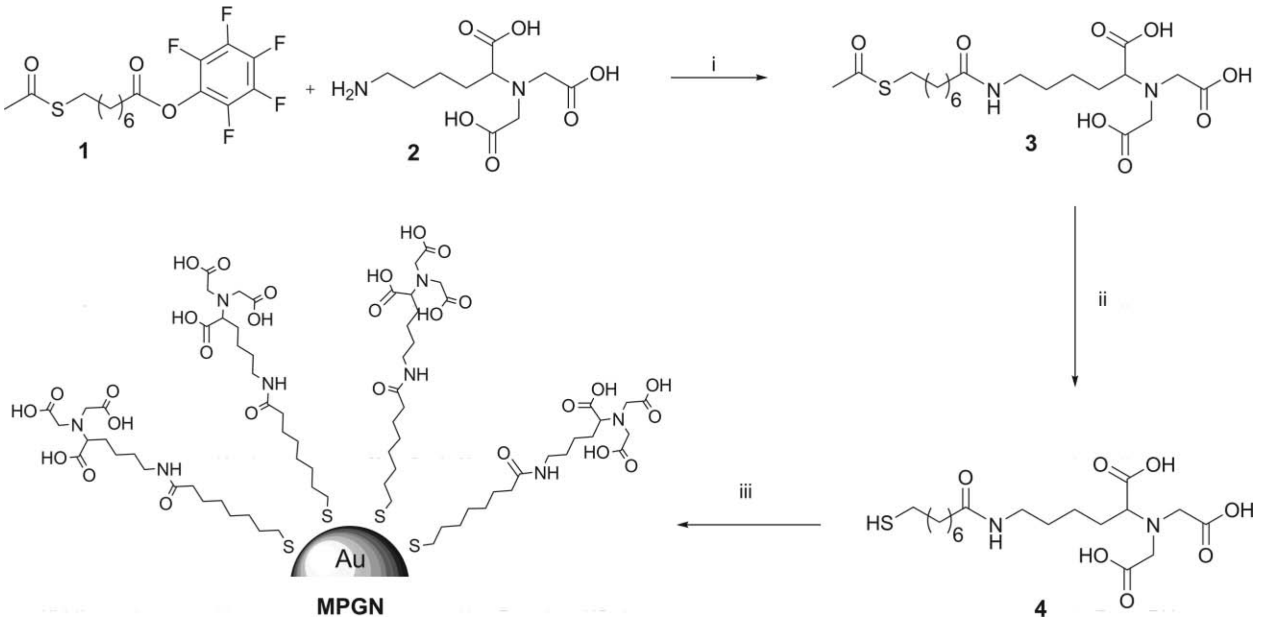
In the past years, multivalent systems have emerged as a new strategy to obtain highly active hydrolytic agents.[13] In such catalysts many active groups, or metal ions, properly assembled can cooperate to give higher activity than an equal amount of the isolated species. Early strategies employed for the realization of such multivalent systems involved the use of surfactant aggregates, such as micelles and liposomes.[14] In particular, Moss and co-workers demonstrated the high activity of Ce(IV) ions in the presence of micelles made by Brij-35 and palmitate.[15] Most recent examples of multivalent hydrolytic agents based on metal complexes (but not Ce(IV)) include polymers,[16] dendrimers,[17] and monolayer protected gold nanoparticles (MPGNs).[18]

MPGNs represent probably the most suitable approach to the realization of multivalent functional systems.[19] The monolayer of organic molecules that protects the nanocluster is made by thiols strongly bound to the gold surface. These thiols may bear at the other end reactive groups that will locate very close to each other thus possibly cooperating in the hydrolytic mechanism. With respect to surfactant aggregates, the non-dynamic nature of MPGNs provides solid bases for effective cooperation between the functional groups.[14] Moreover, preparation of MPGNs is much simpler than that of functional polymers or dendrimers, since the single functional units can be synthesized separately and then self-assembled on the gold core.

In this paper, we report the kinetic investigation of the reactivity of gold nanoparticles bearing Ce(IV) complexes on the coating monolayer. High activity has been observed and the cooperative action of several metal ions is demonstrated.

Results and discussion

Thiol **4** (Scheme 2), which bears a tetradentate policarboxylate ligand, was designed as Ce(IV) binding unit for the preparation of hydrolytic MPGNs.



Scheme 2 Synthesis of **3** coated MPGNs, i) $NaHCO_3$, ii) HCl 3M, iii) MPGNs solution in toluene.

Synthesis of **4** is straightforward (Scheme 2) and involves the coupling of the active ester **1** with N_α,N_α-bis(carboxymethyl)lysine **2**, followed by acid deprotection of the thioacetate. Reference compound **5** (Chart 1) was prepared by acetylation of **2** with acetic anhydride.

Thiol **4** was used to coat the MPGNs following our previously reported two-step procedure for the preparation of water soluble gold nanoparticles.[20] Briefly, nanoparticles are synthesized in toluene by reduction of $HAuCl_4$ with $NaBH_4$ in the presence of tetrabuthylammonium bromide as phase transfer agent and dioctylamine as capping agent. Final nanoparticles size is controlled by the amount of dioctylamine added, which acts as surface stabilizer. Subsequent addition of a thiol, or of a mixture of thiols, to the nanoparticles toluene solution results into the displacement of the amine and the formation of the protecting monolayer.

The **4**-coated nanoparticles were found to be fully soluble in water and stable for several weeks in this solvent. The nanoparticles were characterized for their size by TEM and monolayer purity by ^{1}H-NMR (see ESI†). Size of the metal core of MPGNs resulted to be 2.3 ± 0.8 nm, corresponding to the average formula of $Au_{309}\mathbf{4}_{92}$.[21] Solutions of Ce(IV)-MPGNs were prepared by mixing equimolar (with respect to the **4** units) amounts of freshly prepared $Ce(NH_4)_2(NO_3)_6$ and MPGNs solutions in water. The Ce(IV)-MPGNs solutions so obtained are stable for several days and good reproducibility of the kinetic results was observed over this period.

Hydrolytic activity of Ce(IV)-MPGNs was tested by using bis-*p*-nitrophenyl phosphate as DNA model substrate. To provide a realistic account of the MPGNs reactivity, concentrations were calculated per ligand molecule and so reported in the following text. The reactions were performed at 25 °C and monitored following the absorption of the released *p*-nitrophenoxide at 400 nm. Kinetic profiles obtained indicate that Ce(IV)-MPGNs cause the complete hydrolysis of BNP with the release of two equivalents of *p*-nitrophenol in two subsequent steps. The pseudo-first order rate constants for the two steps were obtained by non-linear regression analysis of the absorbance *vs.* time data (see ESI†). The pseudo-first order rate measured for the cleavage of BNP to *p*-nitrophenyl phosphate (MNP, first step) in the presence Ce(IV)-MPGNs is 3.95×10^{-5} s^{-1}, at a 120 μM concentration of **4** units, 25 °C and pH 7. This value corresponds to a 2.5-million fold rate acceleration over the background reaction.[2] The dependence of the reaction rate over the Ce(IV)-MPGNs concentration is linear in the range explored (25–150 μM of **4** units). The first step of the hydrolytic reaction, the cleavage of BNP to form MNP, is about 4 times faster than the second, the cleavage of the monoester MNP to form inorganic phosphate.[22]

Similarly to the free Ce(IV) ion, the influence of pH on the reactivity is very small (see ESI†). Reaction rate at pH 10 is only double than that at pH 7, indicating that active species are present at all the pH values explored.

Fig. 1 reports the reactivity, relative to the first and the second step of the BNP hydrolysis catalyzed by the Ce(IV)-MPGNs, at different Ce(IV) to **4** units ratios. Inspection of the profiles indicates that the reaction rate levels-off after the addition of one equivalent of Ce(IV), indicating that the most active system are the fully loaded nanoparticles.[23] The sigmoidal profile of the plots suggests a cooperative action of at least two metal ions. Likely, bi- and tri-metallic sites are spontaneously formed at the nanoparticles surface and work as catalytic sites. The inflection points of the plots relative to the first and second steps of the reaction are respectively at 0.5 and 0.8 equivalents of the metal ion. This difference may indicate that the first step of the reaction, the cleavage of BNP to form *p*-nitrophenyl phosphate (MNP), occurs mainly at the bimetallic sites, while hydrolysis of the intermediate MNP is preferentially due to polymetallic sites, which should feature a higher local charge.

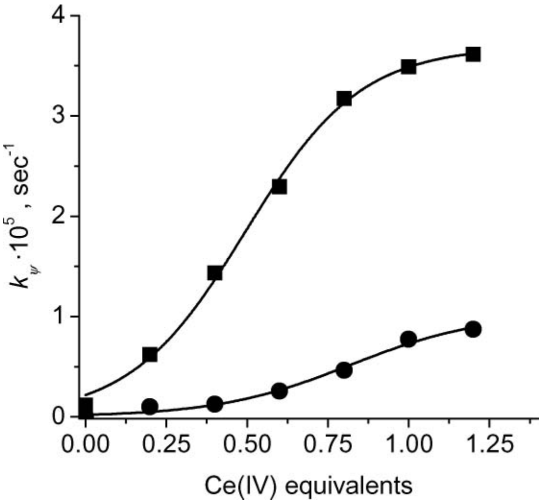


Fig. 1 Rates of BNP cleavage by MPGNs ([**4**] = 5×10^{-5} M) as a function of the equivalents of $Ce(NH_4)_2(NO_3)_6$ added at pH 8.0 (■, release of the first *p*-nitrophenolate; ●, release of the second *p*-nitrophenolate). [CHES buffer] = 5×10^{-3} M, [BNP] = 2×10^{-5} M, 25 °C.

Cooperation between metal units is also supported by the kinetic experiments performed by increasing the substrate concentration in the presence of a fixed amount of Ce(IV)-MPGNs (Fig. 2). A saturation profile is observed, diagnostic of the complexation of the substrate to the nanoparticles before the cleavage.

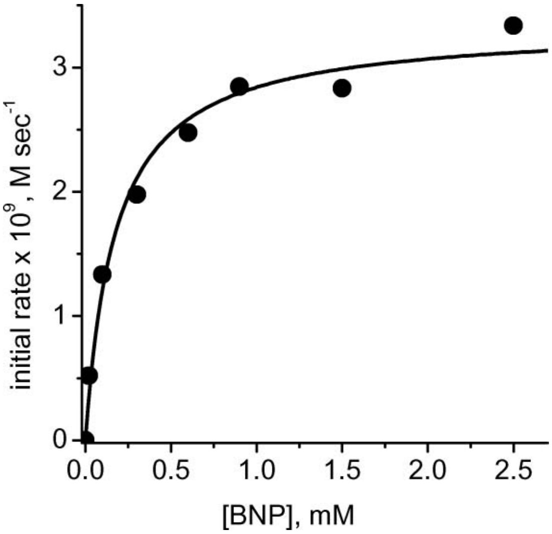


Fig. 2 Rate of BNP cleavage promoted by Ce(IV)-MPGNs as a function of the concentration of BNP. Conditions: [Ce(IV)-MPGNs] = 5.0×10^{-5} M, [buffer] = 5.0×10^{-3} M, pH = 8.0, 25 °C.

The fit of the data with the Michaelis–Menten equation provided $1/K_M$ value of 5700 M^{-1}. Such a high affinity of the

Table 1 BNP cleavage reactivity[a] for different Ce(IV)-based agents: first order rate constants at the concentration reported (k_{obs}); second order rate constant at the pH reported (k_2); second order rate constants relative to the hydroxide promoted reaction (k_{rel})

Entry	Catalyst	Conc./mM	T/°C	k_{obs}/s^{-1}	k_2/s^{-1} M^{-1}	k_{rel}	Ref.
1	OH$^-$	10^{-4}	35	1.1×10^{-10}	2.4×10^{-5}	1	2
2	EDDA-Ce(IV)	0.275	25	1.2×10^{-6}	4.3×10^{-3}[b]	179	7
3	**5**-Ce(IV)	0.15	25	—[c]	—[c]	—[c]	[d]
4	HXTA-Ce(IV)$_2$[e]	0.1	37	1.0×10^{-5}	0.1	4167	12
5	Ce(IV)-micelles	1	37	6.0×10^{4}	0.6 [b]	25000	15
6	Ce(IV)-MPGNs	0.15	25	4.5×10^{-5}	0.3	12500	[d]
7	Ce(IV)-MPGNs	0.15	40	1.1×10^{-4}	0.93	38750	[d]
8	$(NH_4)_2Ce(NO_3)_6$	0.1	25	1.6×10^{-4}	1.6[b]	66667	7

[a] pH 7, unless otherwise stated. [b] Calculated form the data reported in the reference. [c] No reactivity detected. [d] This work. [e] pH 8

reactive sites for the substrate supports their polynuclear nature. In fact, the $1/K_M$ value it is about ten times larger than those reported for lanthanide ions and close to that (5×10^3 M^{-1}) estimated for the $Ce_4(OH)_{15}^+$ hydroxocomplex.[7]

Table 1 lists the reactivity toward BNP of the most representative monometallic, bimetallic and polymetallic Ce(IV) complexes reported. Surprisingly enough, data reported in literature are quite scarce, probably due to the difficulty in obtaining reproducible results. Due to the diversity of conditions used, second order rate constants, when not reported, were calculated from the data available to allow a better comparison. Moreover, data for the reactivity of Ce(IV)-MPGNs at 40 °C have been added, since most of the systems reported have been studied near to this temperature.

All the complexes are less reactive than free $(NH_4)_2Ce(NO_3)_6$, with reference **5**-Ce(IV) being substantially unreactive. As mentioned before, the complexation with electron-donating groups or negatively charged groups likely decreases the Lewis acidity of the metal ion. Moreover, ligands may prevent the formation of the hydroxopolymeric structures that are the active species in the case of free Ce(IV) catalysis. In fact, bimetallic complex of HXTA is more reactive than mononuclear EDDA (Chart 1), but its activity is still far from that of the free ion.

The use of multivalent systems allows a more substantial reactivity rescue. Indeed, Ce(IV)/Brij 35/Palmitate micelles and Ce(IV)-MPGNs are respectively 25000 and 38750 times more active than the hydroxide ion and not far from the reactivity of free Ce(IV). Hence cooperation of multiple Ce(IV) units on an appropriate support, either micelles or nanoparticles, can produce reactivities similar to those of the polynuclear species present in Ce(IV) solution but with better stability and reproducibility.

The finding of a similar reactivity of nanoparticles-based and micellar systems is in contrast with previous studies, which demonstrated that MPGNs coated with Zn(II) complexes where far more reactive than the corresponding micellar system.[18b] In fact, the dynamic nature of surfactant aggregates usually heavily limits the possibility that a cooperative action of the reactive units present in the aggregate may occur.[14] This may not be the case for Ce(IV), whose tendency to form multimeric species is so high that they have been supposed to be present, and catalytically active, even in micellar[15] and vesicular[24] systems.

Conclusion

The results reported in this paper further demonstrate that the self-assembling of reactive units on the surface of monolayer protected gold nanoparticles is an effective and straightforward strategy for the realization of multivalent catalyst with high reactivity. Indeed, the Ce(IV)-MPGNs here studied are able to produce, in the presence of a concentration of Ce(IV) as low as 120 μM, a 2.5 million-fold rate acceleration of the BNP hydrolysis (pH 7, 25 °C), that corresponds to a reduction of the substrate half-life time from 2000 years to a few hours. The MPGNs are 1 to 2 orders of magnitude more reactive than any mono and dinuclear Ce(IV) complex so far reported. The source of such reactivity is the cooperation between the metal ions self-organized on the surface of the nanoparticles. In spite of the above excellent performance, the MPGN-based system is still less active than uncomplexed Ce(IV). This is in sharp contrast with the results previously obtained with Zn(II)-based nanoparticles where free Zn(II) was far less active.[18] The clear explanation is that, contrary to Ce(IV), Zn(II) does not form oligomeric clusters in solution.

Again at variance with other examples, the activity of the nanoparticles-based system is only slightly higher than that of similar micellar system. The great tendency of the Ce(IV) ions to form polymeric species allows probably the cooperation between the metal ions even within a dynamic system as that constituted by a micelle.

Still, the nanoparticles offer other advantages: they may survive in conditions, as high dilution, or environments, such as biological tissues, were micelles can hardly exist. This will allow a much broader applications range. A further development will see the optimization of this promising system for DNA cleavage.

Notes and references

1 (*a*) G. K. Schroeder, C. Lad, P. Wyman, N. H. Williams and R. Wolfenden, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 4052–4055; (*b*) N. H. Williams, B. Takasaki, M. Wall and J. Chin, *Acc. Chem. Res.*, 1999, **32**, 485–493.

2 Spontaneous hydrolysis of BNP at pH 7.0 and 25 °C can be estimated to be 1.6×10^{-11} s^{-1} on the basis of the activation parameters determined at high temperatures: J. Chin, M. Banaszczyk, V. Jubian and X. Zou, *J. Am. Chem. Soc.*, 1989, **111**, 186–190.

3 Selected reviews: (*a*) J. Weston, *Chem. Rev.*, 2005, **105**, 2151–2174; (*b*) N. Strater, W. N. Lipscomb, T. Klabunde and B. Krebs, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 2024–2055.

4 (*a*) F. Mancin and P. Tecilla, in *"Metal Complex-DNA Intractions"*, ed. N. Hadjiliadis and E. Sletten, Wiley, Chichester, 2009, pp. 369–394 and references therein; (*b*) T. Niittymaki and H. Lonnberg, *Org. Biomol. Chem.*, 2006, **4**, 15–25; (*c*) J. R. Morrow and O. Iranzo, *Curr. Opin. Chem. Biol.*, 2004, **8**, 192–200; (*d*) R. Ott and R. Kramer, *Appl. Microbiol. Biotechnol.*, 1999, **52**, 761–767; (*e*) E. L. Hegg and J. N. Burstyn, *Coord. Chem. Rev.*, 1998, **173**, 133–165.

5 M. Komiyama, N. Takeda and H. Shigekawa, *Chem. Commun.*, 1999, 1443–1451.

6 (*a*) M. Komiyama, N. Takeda, Y. Takahashi, H. Uchida, T. Shiiba, T. Kodama and M. Yashiro, *J. Chem. Soc., Perkin Trans. 2*, 1995, 269–274; (*b*) M. Komiyama, T. Shiiba, T. Kodama, N. Takeda, J. Sumaoka and M. Yashiro, *Chem. Lett.*, 1994, 1025–1028.

7 A. L. Maldonado and A. K. Yatsimirsky, *Org. Biomol. Chem.*, 2005, **3**, 2859–2867.

8 J. Sumaoka, T. Igawa, K. Furuki and M. Komiyama, *Chem. Lett.*, 2000, 56–57.

9 (*a*) T. Igawa, J. Sumaoka and M. Komiyama, *Chem. Lett.*, 2000, 356–357; (*b*) A. Kajimura, J. Sumaoka and M. Komiyama, *Carbohydr. Res.*, 1998, **309**, 345–351; (*c*) J. Sumaoka, S. Miyama and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, 1994, 1755–1756.

10 (*a*) Y. Kitamura, J. Sumaoka and M. Komiyama, *Tetrahedron*, 2003, **59**, 10403–10408; (*b*) Y. Kitamura and M. Komiyama, *Nucleic Acids Res.*, 2002, **30**, 102e.

11 (*a*) Y. Xu, Y. Suzuki, T. Lonnberg and M. Komiyama, *J. Am. Chem. Soc.*, 2009, **131**, 2871–2874; (*b*) Y. Miyajima, T. Ishizuka, Y. Yamamoto, J. Sumaoka and M. Komiyama, *J. Am. Chem. Soc.*, 2009, **131**, 2657–2662; (*c*) H. Katada and M. Komiyama, *ChemBioChem*, 2009, **10**, 1279–1288; (*d*) M. Komiyama, Y. Aiba, Y. Yamamoto and J. Sumaoka, *Nat. Protoc.*, 2008, **3**, 655–662; (*e*) T. Lonnberg, Y. Suzuki and M. Komiyama, *Org. Biomol. Chem.*, 2008, **6**, 3580–3587; (*f*) J. Sumaoka, Y. Yamamoto, Y. Kitamura and M. Komiyama, *Curr. Org. Chem.*, 2007, **11**, 463–475.

12 M. E. Branum, A. K. Tipton, S. R. Zhu and L. Que, *J. Am. Chem. Soc.*, 2001, **123**, 1898–1904.

13 L. J. Prins, F. Mancin and P. Scrimin, *Curr. Org. Chem.*, 2009, **13**, 1050–1064.

14 (*a*) S. Bhattacharya and N. Kumari, *Coord. Chem. Rev.*, 2009, **253**, 2133–2149; (*b*) F. Mancin, P. Scrimin, P. Tecilla and U. Tonellato, *Coord. Chem. Rev.*, 2009, **253**, 2150–2165; (*c*) J. Zhang, X. G. Meng, X. C. Zeng and X. Q. Yu, *Coord. Chem. Rev.*, 2009, **253**, 2166–2177.

15 K. Bracken, R. A. Moss and K. G. Ragunathan, *J. Am. Chem. Soc.*, 1997, **119**, 9323–9324.

16 (*a*) G. Zaupa, L. J. Prins and P. Scrimin, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 3816–3820; (*b*) J. Suh, *Acc. Chem. Res.*, 2003, **36**, 562–570.

17 (*a*) G. Zaupa, P. Scrimin and L. J. Prins, *J. Am. Chem. Soc.*, 2008, **130**, 5699–5709; (*b*) M. Martin, F. Manea, R. Fiammengo, L. J. Prins, L. Pasquato and P. Scrimin, *J. Am. Chem. Soc.*, 2007, **129**, 6982–6983.

18 (*a*) R. Bonomi, F. Selvestrel, V. Lombardo, C. Sissi, S. Polizzi, F. Mancin, U. Tonellato and P. Scrimin, *J. Am. Chem. Soc.*, 2008, **130**, 15744–15745; (*b*) F. Manea, F. B. Houillon, L. Pasquato and P. Scrimin, *Angew. Chem., Int. Ed.*, 2004, **43**, 6165–6169.

19 L. Pasquato, P. Pengo and P. Scrimin, *Supramol. Chem.*, 2005, **17**, 163–171.

20 F. Manea, C. Bindoli, S. Polizzi, L. Lay and P. Scrimin, *Langmuir*, 2008, **24**, 4120–4124.

21 M. J. Hostetler, J. E. Wingate, C. J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans and R. W. Murray, *Langmuir*, 1998, **14**, 17–30.

22 Such lower reactivity toward MNP is not umprecedented: different selectivities of Ce(IV) complexes toward phosphate diesters and monoesters, depending on subtle differences in the ligand structure, have been reported, see ref.15.

23 At higher Ce(IV) concentrations a further reactivity increase is observed due to the formation of Ce(IV) hydroxoclusters, see ESI.

24 (*a*) R. A. Moss, B. D. Park, P. Scrimin and G. Ghirlanda, *J. Chem. Soc., Chem. Commun.*, 1995, 1627–1628; (*b*) P. Scrimin, P. Tecilla, R. A. Moss and K. Bracken, *J. Am. Chem. Soc.*, 1998, **120**, 1179–1185; (*c*) P. Scrimin, S. Caruso, N. Paggiarin and P. Tecilla, *Langmuir*, 2000, **16**, 203–209.