Resin-supported catalytic dendrimers as multivalent artificial metallonucleases

Giovanni Zaupa, Leonard J. Prins *, Paolo Scrimin *

Department of Chemical Sciences, University of Padova and CNR ITM, Padova Section, Via Marzolo 1, 35131 Padova, Italy

ABSTRACT

Tentagel resin was functionalized with dendrons containing 4 and 8 triazacyclononane ligands able to complex the Zn^{II} metal ion. The supported dendritic metallo-complexes showed enzyme-like behaviour in the cleavage of HPNPP, a model substrate for RNA. The obtained Michaelis–Menten parameters were in excellent agreement with those obtained for the identical catalysts in solution. Diffusion studies have revealed the upper limit for the rate constants that can be assessed under these conditions.

© 2009 Elsevier Ltd. All rights reserved.

Constituting the backbone of the oligonucleotides DNA and RNA, the phosphate diester bond is of crucial biological importance. Its impressive high stability against hydrolytic cleavage is evidenced by the fact that estimates for the half-life for the cleavage of a single P–O bond at 25 °C at pH 7 range from hundreds of thousands to hundreds of millions of years.[1] Equally impressive is the ability of nucleases to cleave this bond within seconds. Many of these enzymes contain multiple metal ions (mainly Ca(II), Mg(II), and Zn(II)) in the active site, which play a fundamental role in the catalytic pathway.[2] Mechanistic studies have shown that several metal ions can accelerate phosphate bond cleavage in a cooperative manner. Currently, there is a strong interest in the development of artificial metallonucleases that operate in a mechanistically similar way.[3] For the purpose of bringing multiple metal ions together, multivalent scaffolds ranging from tripodal molecules,[4,5] dendrimers,[6] and polymers[7] to Au-nanoparticles[8] and also resins[9] have been extensively used, occasionally yielding catalysts with excellent properties. Recently, we have observed the cooperative action between two Zn(II) ions located at the periphery of a dendrimer giving rise to a positive dendritic effect.[6] Subsequent model studies have shown that this dendritic effect is an intrinsic consequence of clustering functional units together in a multivalent structure.[10]

The next challenge for this type of catalysts is the addition of functional groups to the catalytic site that can further boost the catalytic performance of the system.[11] The development of such heterofunctionalized multivalent systems requires new synthetic protocols that should be fast, straightforward, and reliable.[12] From this perspective, the functionalization of dendrimers on solid support is highly attractive as it benefits from the advantages of solid phase synthesis.[13] Furthermore, a direct on-bead screening of catalytic activity would allow the implementation of automated protocols.[14] Here, we show the straightforward synthesis of metallodendrimers on solid support and show that the obtained catalytic parameters of the immobilized systems are identical to the homogeneous catalysts. Diffusion studies illustrate the upper limit for the rate constants that can be measured for the heterogeneous catalysts under these conditions.

Resin-supported dendrimers were obtained in a single step by reacting commercially available Tentagel MAP 4 and MAP 8 resin (mixed polystyrene-polyethyleneglycol beads functionalized with a lysine-based dendrimer backbone) with triazacyclonane (TACN)-derivative **1** (Chart 1).[15] A complete reaction of all terminal NH_2-groups was confirmed by the negative results of both the Kaiser-test and a stain with fluorescein thioisocyanate. Before using the dendrimer resins, the BOC-protecting groups of TACN were removed by treatment with 30% CF_3COOH in dichloromethane. For the kinetic studies, the dendrimer resins were dispersed in 96 well microtiter plates by taking volumes of a homogeneous resin suspension of a known concentration.[16] Although reported,[17] the precision of this procedure for resin dispersion was determined by scanning single wells and comparing the number of beads with those calculated (2.86 beads/g resin). Typically, an error in the order of 10% was found. Catalytic studies were performed using 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP) as a substrate, which is the standard model of an RNA-phosphodiester (Fig. 1a). All kinetic studies were performed in water at 40 °C buffered with HEPES (16 mM) at pH 7.0, measuring the release of the *p*-nitrophenolate ion at 405 nm.

* Corresponding authors. Tel.: +39 049 8275276; fax: +39 049 8275239 (P.S.).
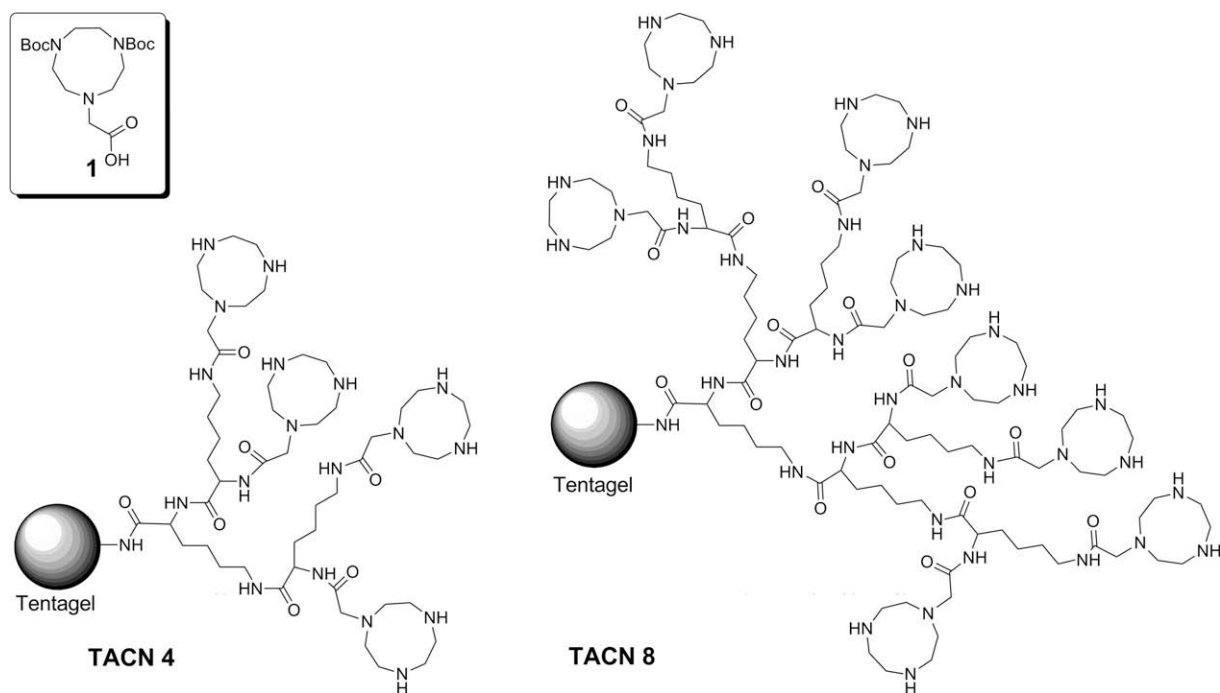 E-mail addresses: leonard.prins@unipd.it (L.J. Prins), paolo.scrimin@unipd.it (P. Scrimin).

Chart 1. Tentagel TACN 4 and TACN 8 resin obtained in a single step by reacting Tentagel MAP 4 (0.6 mmol NH$_2$/g) and MAP 8 resin (0.8 mmol NH$_2$/g) with TACN-derivative **1** (PyBOP, NMM, DMF).
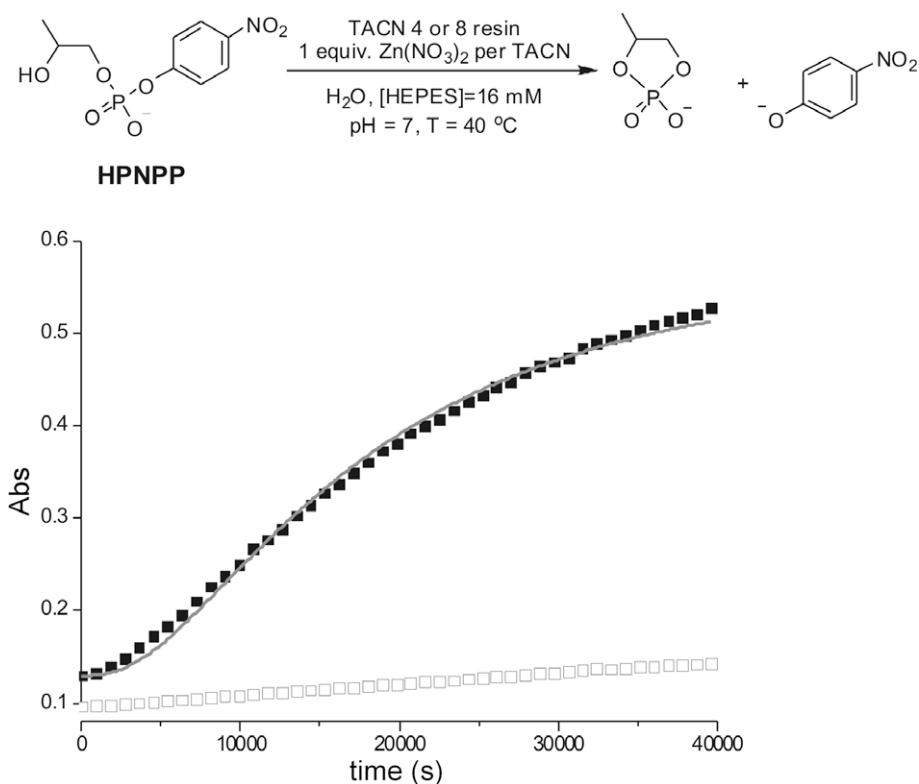

Figure 1. (a) General reaction conditions for the transesterification of HPNPP and (b) a typical kinetic run illustrating the catalytic activity of the TACN 4 resin (■) (conditions: [TACN] = [Zn(NO$_3$)$_2$] = 1.3 × 10^{-4} M; [HPNPP] = 1.6 × 10^{-4} M). Catalysis by the same amount of Zn(II) in the absence of resin is given as illustration (□). The solid line is the best fit to the model shown in the inset of Figure 3.

In a first set of studies the catalytic activity of both TACN 4 and TACN 8 resins in the presence of increasing amounts of Zn(II) was studied. A typical example of a kinetic run is given in Figure 1b, because it indicates two important features. First, the resins are catalytically active, evidenced by the fact that the same concentration of Zn(II) in the absence of resin induces a much lower cleavage rate. Second, contrary to identical studies in homogeneous systems,[10] the kinetics follow a sigmoidal profile. Also taking into

account that the total increase in absorbance is only 80% of that anticipated for the amount of substrate present (A_{meas} = 0.6, A_{calc} = 0.8), it indicates that substrate/product diffusion and partitioning between solid and solution phase are relevant issues. These issues will be addressed later. Plotting the measured initial rate (discarding the initial fragment of each titration) as a function of the amount of Zn(II) added shows that a maximum activity is reached when TACN and Zn(II) are present in an equimolar amount (Fig. 2a).

Next, the saturation behaviour of the resins was examined by measuring the initial rates as a function of the amount of added substrate (Fig. 2b). The obtained profiles were fitted using the Michaelis–Menten equation yielding the values for k_{cat} and K_M given in Table 1. It should be noted that the catalyst concentration

Table 1
Michaelis–Menten parameters for the TACN 4 and TACN resins and the analogous dendrons in solution[a]

Catalyst	k_{cat}[b] ($\times 10^{-4}$ s^{-1})	K_M (mM)	k_{cat}/K_M (M^{-1} s^{-1})
TACN 4			
Solution	4.1	1.0	0.41
Resin	2.7	0.7	0.39
TACN 8			
Solution	4.1	0.9	0.46
Resin	3.5	0.4	0.88

[a] As reported previously.[10]
[b] k_{cat} refers to the actual concentration of the catalytic unit TACN, not the dendron concentration.
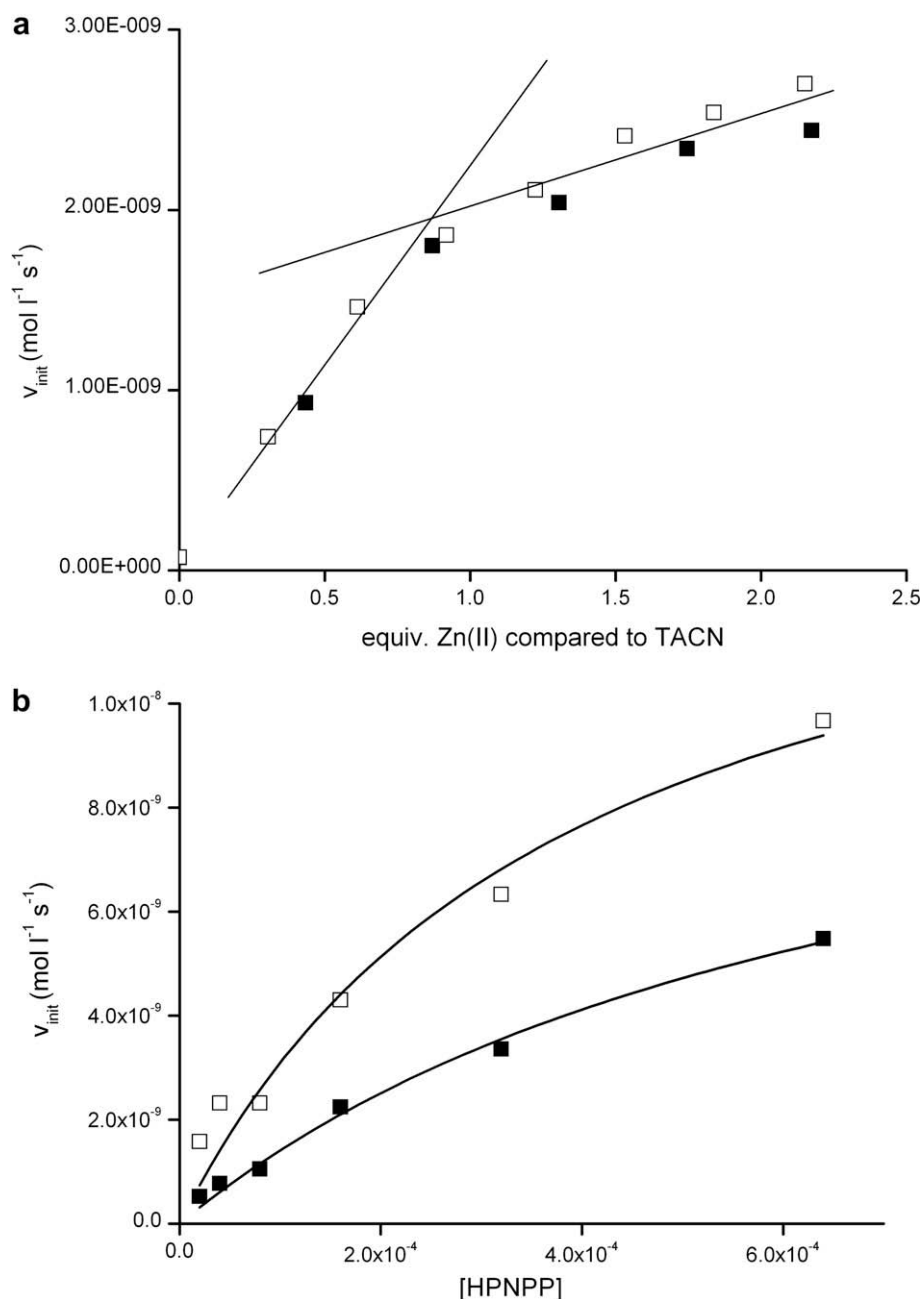

Figure 2. (a) Initial rates for the cleavage of HPNPP by the TACN 4 (■) and TACN 8 (□) resin as a function of the amount of Zn(II) added. Conditions: [TACN] = 4 × 10^{-5} M (■) or 3 × 10^{-5} M (□); [HPNPP] = 1.6 × 10^{-4} M; [HEPES] = 1.6 × 0^{-2} M. The solid lines are trend lines that illustrate the change in slope when TACN and Zn(II) are present in a 1:1 ratio. (b) Saturation profiles for the TACN 4 (■) and TACN 8 (□) resins. The solid lines represent the best fits to the Michaelis–Menten equation. Conditions: [TACN] = 1.2 × 10^{-4} M; [Zn(II)] = 1.2 × 10^{-4} M; [HEPES] = 1.6 × 10^{-2} M.

refers to the concentration of the TACN·Zn(II) complex enabling a direct comparison between the catalytic activity of this unit in the TACN 4 and the TACN 8 resins. As comparison, also the values previously obtained for the analogous dendrons in solution are given.[10] Analysis of the data shows a better catalytic performance for the TACN 8 resin with respect both to the TACN 4 resin and the corresponding dendrons in solution, which is reflected by a 2.4-fold increase in the second order rate constant k_{cat}/K_M, which is for a large part attributable to stronger substrate binding (lower K_M). The obtained data are in good correlation with the solution data, both in absolute and relative manner. A higher substrate affinity is observed for the resin-bound dendrons, which presumably originates from the resin itself (see below).

The good correspondence between the solution and solid phase data is promising for the application of this protocol for the rapid synthesis and screening of heterofunctionalized multivalent

catalysts. Nonetheless, the observation of diffusion related processes and product partitioning during the kinetic studies made us study these phenomena in more detail. p-Nitrophenol was added to a well containing a known quantity of the TACN 4 resin in the presence of 1 equiv of Zn(II) per TACN. The measured absorbance at 405 nm was followed in time showing a gradual decrease to around 80% of the initial value (from 0.9 to 0.72) (Fig. 3). The decrease in absorbance originates from a decreased p-nitropheno-late concentration in solution resulting from a sequestering by the resin, also visually observed by an intense yellow colouring of the resin beads. The curve was fitted to a simple model assuming pseudo-first order diffusion kinetics yielding rate constants of 1.6×10^{-4} and 7.9×10^{-4} s^{-1} for the inward and outbound pathway, respectively. The partitioning coefficient of 0.2 (k_{in}/k_{out}) corresponds nicely to the previous observation that the measured absorbance amounts only to 80% of the expected absorbance.
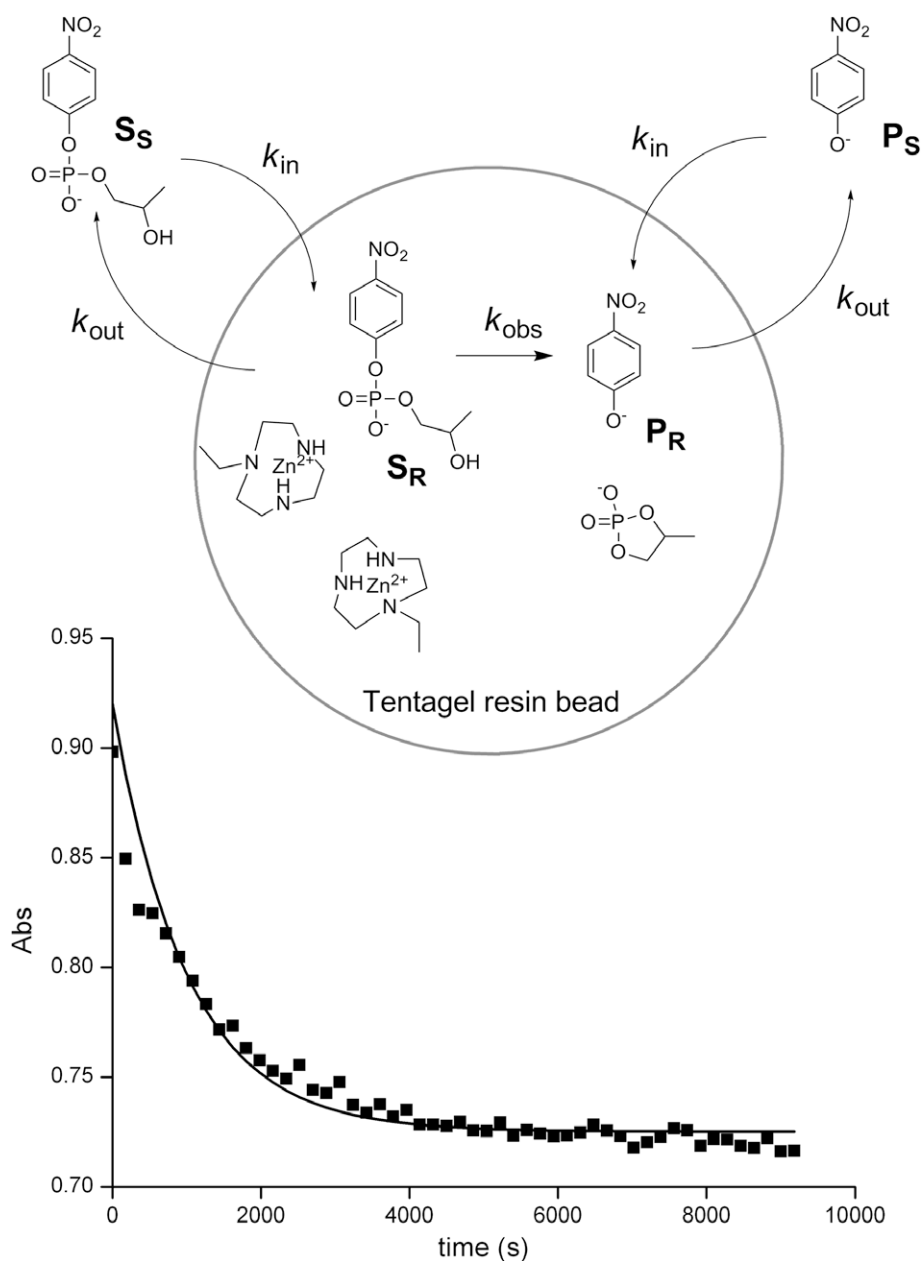

Figure 3. Kinetic profile obtained for the diffusion of p-nitrophenol into the TACN 4 resin (equilibrium between **PS** and **PR** as shown above). Conditions: [PNP] = 1.6×10^{-4} M; [TACN 4 resin] = 150 beads/250 µl; [Zn(II)] = 1.3×10^{-4} M. The solid line is the best fit to a model assuming pseudo-first order conditions yielding values for k_{in} and k_{out} of 1.6×10^{-4} and 7.9×10^{-4} s^{-1}, respectively.

Assuming that the substrate HPNPP has similar diffusion rates, the kinetic profile of Figure 1 was re-fitted taking into account substrate diffusion into the resin (according to the model in the inset of Fig. 3). Considering the simplicity of this model, an excellent fit was obtained, giving a k_{obs} value of $1.4 \times 10^{-4} \, s^{-1}$. Being lower than the pseudo-first order rate constants obtained for the diffusion process, this value evidences that the catalytic conversion is the rate determining step.[18] Nonetheless, the margins for catalyst improvement appear relatively small before diffusion becomes the limiting step. Such problems may be prevented by using macro pore resins specifically designed for rapid diffusion.[19]

In summary, we have established a straightforward protocol for the synthesis and screening of multivalent catalysts on solid support. The measured Michaelis–Menten parameters are in good accord with those found for the identical catalysts in solution. It has been shown that accessibility of the multivalent catalysts in the resin is an important parameter for the applicability of this approach.

Acknowledgments

References and notes

1. Williams, N. H.; Takasaki, B.; Wall, M.; Chin, J. *Acc. Chem. Res.* **1999**, *32*, 485.
2. Wilcox, D. E. *Chem. Rev.* **1996**, *96*, 2435.
3. Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Chem. Commun.* **2005**, 2540.
4. Yashiro, M.; Ishikubo, A.; Komiyama, M. *Chem. Commun.* **1997**, 83.
5. Komiyama, M.; Kina, S.; Matsumura, K.; Sumaoka, J.; Tobey, S.; Lynch, V. M.; Anslyn, E. *J. Am. Chem. Soc.* **2002**, *124*, 13731.
6. Martin, M.; Manea, F.; Fiammengo, R.; Prins, L. J.; Pasquato, L.; Scrimin, P. *J. Am. Chem. Soc.* **2007**, *129*, 6982.
7. Bonora, G. M.; Drioli, S.; Felluga, F.; Mancin, F.; Rossi, P.; Scrimin, P.; Tecilla, P. *Tetrahedron Lett.* **2003**, *44*, 535.
8. Manea, F.; Houillon, F. B.; Pasquato, L.; Scrimin, P. *Angew. Chem., Int. Ed.* **2004**, *43*, 6165.
9. Bodsgard, B. R.; Burstyn, J. N. *Chem. Commun.* **2001**, 647.
10. Zaupa, G.; Scrimin, P.; Prins, L. J. *J. Am. Chem. Soc.* **2008**, *130*, 5699.
11. Natale, D.; Mareque-Rivas, J. C. *Chem. Commun.* **2008**, 425.
12. Zaupa, G.; Martin, M.; Prins, L. J.; Scrimin, P. *New J. Chem.* **2006**, *30*, 1493.
13. Wells, N. J.; Basso, A.; Bradley, M. *Biopolymers* **1998**, *47*, 381.
14. Berkessel, A.; Herault, D. A. *Angew. Chem., Int. Ed.* **1999**, *38*, 102.
15. Commercially available MAP-resin was treated twice with piperidine (20%) in DMF for 15 min to remove the Fmoc-protecting group. Then the resin was washed with DMF ($3\times$) and CH_2Cl_2 ($3\times$). 2-(Di-Boc)TACN acetic acid (6 equiv with respect to the amino groups) was dissolved in CH_2Cl_2/DMF (1:1), and activated with PyBOP (8 equiv) for 10 min in the presence of N-methylmorpholine (20 equiv). The solution was added to the resin and after 4 h the resin was filtered and washed with DMF ($3\times$) and CH_2Cl_2 ($3\times$). The completion of the coupling was confirmed by the Kaiser test and reaction with fluorescein thioisocyanate. Finally, the Boc-protecting group was cleaved by treatment of the resin with trifluoroacetic acid in CH_2Cl_2 (30%) for three hours.
16. The concentration of TACN groups in the kinetic experiments was obtained by the formula:

$$[TNCN] = \frac{n/N \cdot \text{loading}}{V}$$

    where n is the n° of beads per well, N the number of beads per gram of resin (from provider), loading is the number of moles per gram of resin (from provider), V the volume of the solution.
17. Lebl, M.; Pokorny, V.; Krchnak, V. *J. Comb. Chem.* **2005**, *7*, 42.
18. It should be emphasised that these values are pseudo-first order rate constants and cannot be directly compared to the k_{cat}-value for the catalytic conversion.
19. Basso, A.; Braiuca, P.; De Martin, L.; Ebert, C.; Gardossi, L.; Linda, P.; Verdelli, S.; Tam, A. *Chem. Eur. J.* **2004**, *10*, 1007.