Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

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

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1. Experimental Procedures.

General: Solvents were purified by standard methods. All commercially available reagents and substrates were used as received. TLC analyses were performed using Merck 60 F_{254} precoated silica gel glass plates. Column chromatography was carried out on Macherey-Nagel silica gel 60 (70-230 mesh). NMR spectra were recorded using a Bruker AC250F spectrometer operating at 250 MHz for ^{1}H and 62.9 MHz for ^{13}C and a Bruker AV300 operating at 300 MHz for ^{1}H and 121.5 MHz for ^{31}P. Chemical shifts are reported relative to internal Me_4Si. Multiplicity is given as follow: s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, br = broad peak. ESI-MS mass spectra were obtained with an Agilent Technologies LC/MSD Trap SL mass spectrometer. UV-Visible spectra and kinetic traces were recorded on Perkin Elmer Lambda 16 and Lambda 45 spectrophotometers equipped with thermostated multiple cell holders. $Ce(NH_4)_2(NO_3)_6$ was an analytical grade product. The buffer components were used as supplied by the manufacturers: 2-morpholinoethanesulfonic acid (MES, Fluka), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma), 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPES, Sigma), 2-[N-cyclohexylamino]ethanesulfonic acid (CHES, Sigma),), 3-[cyclohexylamino]1-propanesulfonic acid (CAPS, Sigma). The bis-*p*-nitrophenyl phosphate sodium salt (BNP), *p*-nitrophenyl phosphate disodium salt (MNP) and N_a,N_a-Bis(carboxymethyl)-L-Lysine were Aldrich products, used as received.

Kinetic Measurements. The MPGNs and Ce(IV)-MPGNs are fully soluble in water. Concentration of metal binding units (**4**) in MPGNs stock solutions in water were determined by ^{1}H-NMR analysis using acetonitrile as internal standard. Concentrations obtained are in good agreement with the results of Ce(IV) dependant kinetic experiments.

The reactions were started by adding 10 μL of a $2.0 \cdot 10^{-3}$ M solution of substrate (BNP or MNP) to a 2-mL solution containing the appropriate buffer (0.05 M), $Ce(NH_4)_2(NO_3)_6$, MPGNs, and monitored by following the absorption of *p*-nitrophenoxide at 400 nm. Reactions where followed up to at least 90% substrate cleavage and the rate constants were obtained by non-linear regression analysis of the absorbance *vs.* time data.

Data analysis. The kinetic profiles revealed the subsequent release of both the *p*-nitrophenoxide groups, the first process being faster than the second. As a consequence, the data were fitted according to the integrated equation 1 for two consecutive reactions:

$$A = A_{\inf}\left(1 + \frac{k_{\psi}e^{-k_b t} + (k_{\psi} - 2k_2)e^{-k_{\psi} t}}{2(k_b - k_{\psi})}\right) \quad (1)$$

where k_{ψ} is the pseudo-first order rate constant for BNP cleavage and k_b is the pseudo-first order rate constant for subsequent cleavage of MNP. Reliability of the k_b values determined was confirmed by comparison with the ones obtained in the MNP cleavage experiments. The fit error on the rate constants was always less than 5%.

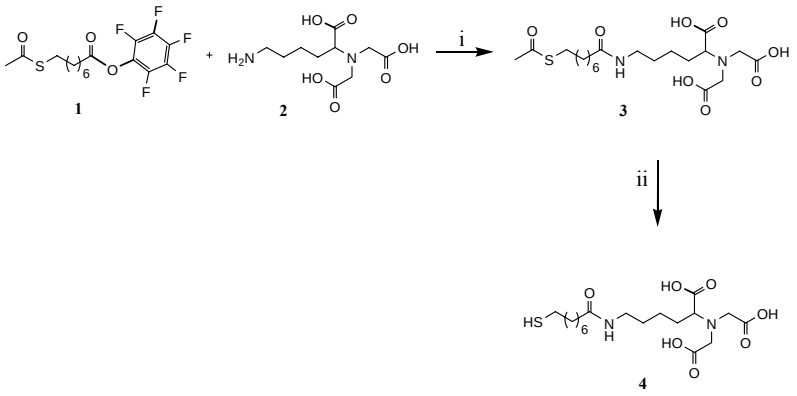
Kinetic experiments with variable BNP concentrations where fitted according to the Michealis-Menten equation (2):

$$v = \frac{k_{\max}[BNP]}{K_M + [BNP]} \quad (2)$$

where $k_{\max}$ is the limiting reaction rate in the experimental conditions and K_M is the apparent dissociation constant of the substrate-catalyst complex.

2. Synthesis

Thiol **4** was prepared according to the following scheme:



2.1 8-thioacetyl pentafluorophenyl octanoate (1)

8-Bromooctanoic acid (2.00 g, 8.96 mmol) was dissolved in acetone (60 mL) and potassium thioacetate (2.25 g, 19.72 mmol) was added. The mixture was refluxed for 48 hours, the solvent was evaporated and the solid residue dissolved in CH_2Cl_2 (20 mL). The organic solution was extracted with water (5 x 20 mL) and dried with Na_2SO_4. After solvent evaporation 1.917 g (98%) of 8-(thioacetyl)-octanoic acid were obtained as an orange oil. ^{1}H-NMR ($CDCl_3$, 250 MHz), δ: 2.83 (t, 2H, 7 Hz), 2.31 (t, 2H, 7 Hz), 2.29 (s, 3H), 1.59 (m, 4H), 1.30 (m, 6H).

8-(Thioacety)-octanoic acid (1.917 g, 8.78 mmol) and pentafluorophenol (2.101 g, 11.414 mmol) were dissolved in CH_2Cl_2 (60 mL) and *N*-(3-Dimethylaminopropyl)-*N′*-ethyl-carbodiimide hydrochloride (EDC, 2.188 g, 11.414 mmol) was added. The mixture was stirred for 12 hours under nitrogen. The organic solution was extracted with water (5 x 20 mL) and dried with Na_2SO_4. After solvent evaporation, the crude product was purified by flash chromatography (silica gel, eluent: CH_2Cl_2/Petroleum Ether 1:1). 1.344 g (39%) of **1** were obtained as a yellow oil.

^{1}H-NMR ($CDCl_3$, 250 MHz), δ: 2.82 (t, 2H, 7 Hz), 2.62 (t, 2H, 7 Hz), 2.27 (s, 3H), 1.72 (qn, 2H, 7 Hz), 1.54 (qn, 2H, 7 Hz), 1.34 (m, 6H).

2.2 N-[N_α,N_α-bis(carboxymethyl)-L-Lysine]8-(Acetylthio)octylamide (3)

To a water solution of bis(carboxymethyl)-L-Lysine (**2**) (0.500 g, 1.79 mmol) and 0.501 g $NaHCO_3$ (5.96 mmol), a solution of 8-thioacetyl pentafluorophenyl octanoate (**1**) (0.578 g, 1.5 mmol) in acetone/ethanol 1:1 (11 mL) was added under stirring. After 30 hours, the ethanol was removed under reduced pressure and additional 5 mL water were added. The white solid present in the mixture was filtered and discarded. The filtrate was acidified with 1 M HCl to pH = 3 and the resulting precipitate was collected, washed with cold water (3 x 5 mL) and acetone (2 mL), and dried under vacuum to give 0.180 g of a yellowish solid (Yield: 26%).

^{1}H-NMR (DMSO, 300 MHz), δ: 7.67 (br, 1H), 3.32 (m, 5H), 2.98 (q, 2H, 6 Hz), 2.81 (t, 2H, 7 Hz), 2.31 (s, 3H), 2.01 (t, 2H, 7 Hz), 1.48 (m, 6H), 1.25 (m, 10H).

^{13}C-NMR (CD_3OD, 62.9 MHz, ^{1}H decoupled), δ: 197.75, 176.40, 176.06, 175.98, 66.79, 55.51, 40.19, 37.21, 30.81, 30.67, 30.21, 30.07, 29.95, 29.76, 27.08, 24.90.

ESI-MS (m/z): 463.2 [100%, $M+H^+$], 485.2 [42%, $M+Na^+$].

2.3 N-[N_α,N_α-bis(carboxymethyl)-L-Lysine]8-Mercapto-octylamide (4)

0.020 g (0.043 mmol) of **3** were dissolved in water (1.5 mL). A 6 M HCl solution in water (1.5 mL) was added and the mixture was stirred at 60 °C for 3 hours. The reaction mixture was allowed to cool and the solvent evaporated to obtain 0.019 g (99%) of **4** as a yellow oil.

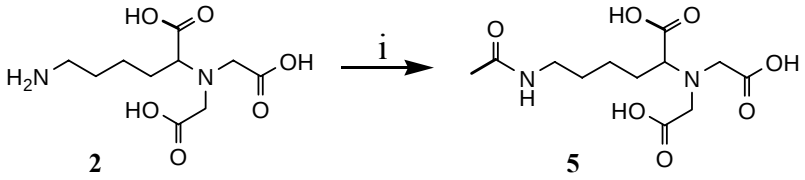
^{1}H-NMR (CD_3OD, 250 MHz), δ: 4.25-4.50 (m, 5H), 3.45 (br, 2H), 2.48 (t, 2H, 7 Hz), 2.43 (t, 2H, 7 Hz), 2.06 (br, 2H), 1.66 (m, 8H), 1.35 (m, 6H).

^{13}C-NMR (CD_3OD, 62.9 MHz, ^{1}H decoupled), δ: 178.24, 170.36, 168.92, 68.21, 55.29, 41.12, 35.89, 35.16, 30.12, 29.79, 29.32, 29.24, 27.90, 27.25, 25.05, 24.74.

ESI-MS (m/z): 421.3 [100%, $M+H^+$], 443.2 [12%, $M+Na^+$].

Elemental analysis, calcd. for $C_{19}H_{34}N_2O_7S\cdot HCl$ (471.00): C 48.45, H 7.49, N 5.95, S 6.81; found: C 48.52, H 7.56, N 5.82, S 6.88 %.

Compound **5** was prepared according to the following scheme:



2 **5**

2.5 N-Acetyl-bis(carboxymethyl)-L-Lysine (5)

To a water solution (5 mL) of bis(carboxymethyl)-L-Lysine (**2**) (0.100 g, 0.357 mmol) and 0.247 g of K_2CO_3 (1.785 mmol), a solution of acetic anhydride (0.415 g, 4.070 mmol) in acetone/ethanol 1:10 (11 mL) was added under stirring. After 30 hours, the ethanol was removed under reduced pressure and 10 mL of HCl 1M were added. The water was evaporated to give 0.302 g of white solid (Yield: 78%).

^{1}H-NMR (D_2O, 300 MHz), δ: 4.49 (m, 5H), 3.35 (t, 2H, 7 Hz), 2.17 (m, 2H), 1.71 (m, 4H).

^{13}C-NMR (CD_3OD, 62.9 MHz, ^{1}H decoupled), δ: 174.88, 171.18, 169.83, 68.04, 55.33, 39.91, 28.67, 27.37, 23.82, 22.71.

ESI-MS (m/z): 304.3 [62%, $M+H^+$], 342.0 [100%, $M+K^+$].

3. Synthesis and characterisation of MPGNs

All the glassware used in the MPGN preparation were washed with aqua regia and rinsed with distilled water. $HAuCl_4$ is strongly hygroscopic and was weighted in a dry-box.

A solution of $HAuCl_4 \cdot H_2O$ (100 mg, 0.281 mmol) in water (7 mL) was extracted with a solution of tetraoctylammonium bromide (2.74 g, 5.01 mmol) in N_2 purged toluene (125 mL divided in 3 portions). To the resulting reddish-orange organic solution, a second solution of tetraoctylammonium bromide (2.74 g, 5.01 mmol) and dioctylamine (3.36 g, 13.9 mmol) is added (the amount of dioctylamine was calculated in order to obtain 2 nm nanoparticles[1]). The mixture was vigorously stirred under N_2 for 30 min. During this period of time the colour of the mixture faded. A solution of $NaBH_4$ (93.0 mg, 2.46 mmol) in H_2O mQ (4.20 mL) was then rapidly added. The color of the solution turned rapidly to black due to nanoparticles formation. After 5 hours of stirring, the aqueous layer was removed.

To a portion (125 mL) of the above nanoparticle solution, 3.0 mL of a solution of 0.187 mmol of thiol **3** in isopropanol were rapidly added. The reaction mixture was stirred vigorously overnight and the solvent was evaporated. The nanoparticles were dissolved in 3 mL of methanol and passed through a Sephadex LH-60 column using methanol as eluent. The methanol was evaporated and the residue (17.5 mg) was redissolved in water.

TEM analysis (Figure S1) yields an average diameter for the MPGNs of 2.3±0.8 nm. Such size corresponds, on the basis of literature data to the general formula $Au_{301}RS_{92}$ (the calculated diameter corresponding to this formula is 2.2 nm),[2] where RS indicate the thiols molecule forming the protecting monolayer. NMR analysis (Figure S2) indicates monolayer formation (broadening of all bands), as confirmed by diffusion filtered experiments (not shown).

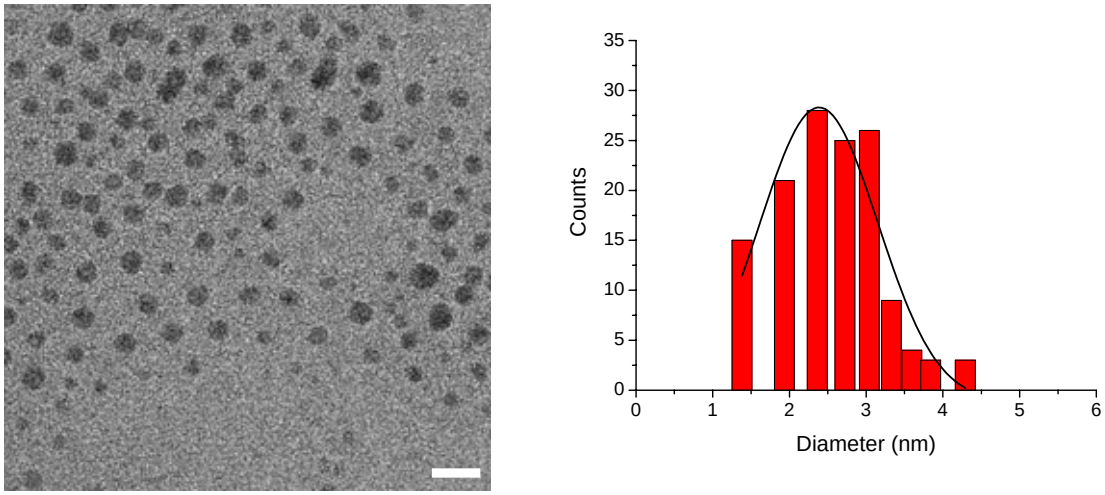


Figure S1: TEM image of the MPGNs (the bar corresponds to 6 nm) and size distribution: average diameter = 2.3 nm (σ = 0.8 nm).

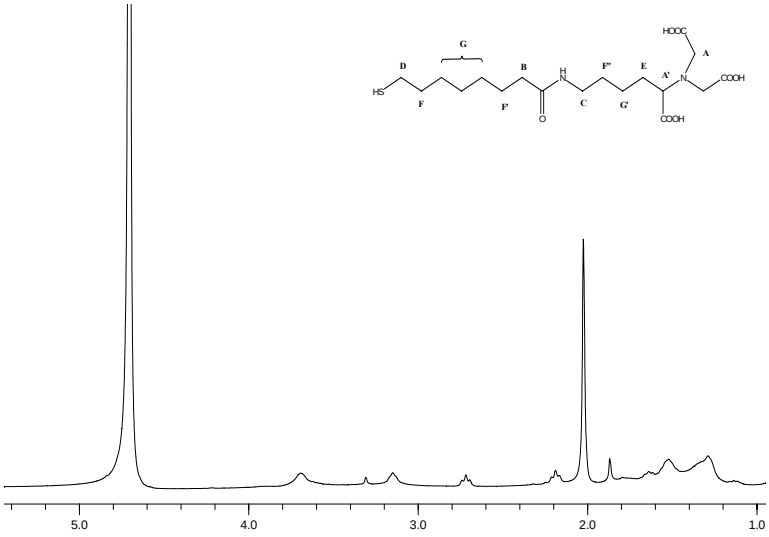


Figure S2: ^{1}H-NMR (300 MHz) spectrum of the MPGNs in CD_3OD. Peak used to determine the ligand concentration is CH_3CN (AcN).

4. pH dependant and catalyst concentration dependant kinetic experiments

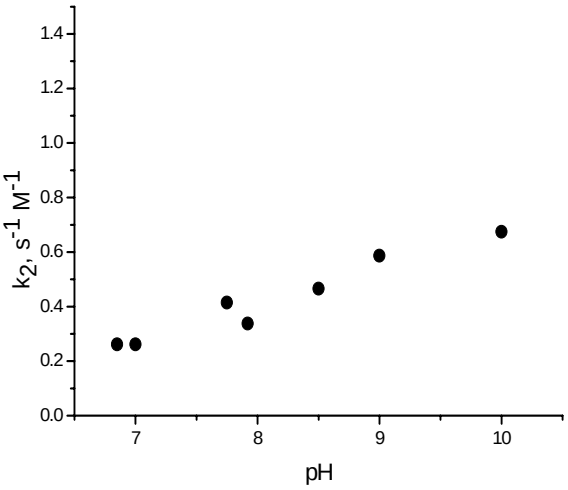


Figure S3. pH dependence of the second-first order rate constants for the reaction between BNP and Ce(IV)-MPGNs. Conditions: [Ce(IV)-MPGN] = 5.0×10^{-5} M, [buffer] = 5.0×10^{-3} M, [BNP] = 2×10^{-5} M, 25 °C.

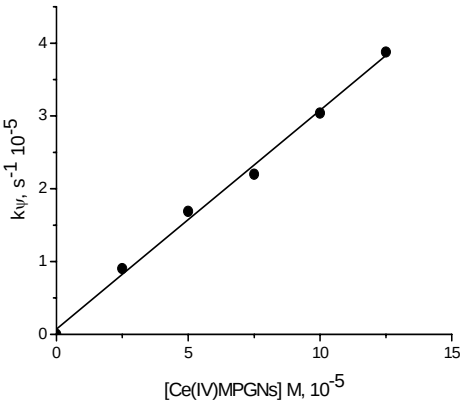


Figure S4. Pseudo-first order rate constant for the reaction between BNP and Ce(IV)-MPGNs as a function of the catalyst concentration. Conditions: [buffer] = $5.0\cdot10^{-3}$ M, [BNP] = $2\cdot10^{-5}$ M, pH 8, 25 °C.

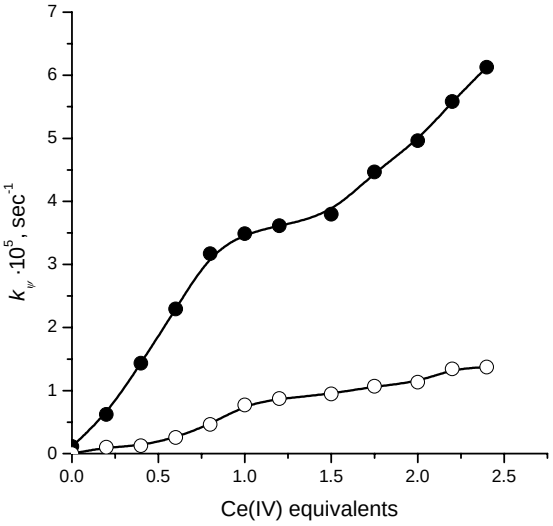


Figure S5 Rates of BNP cleavage by MPGNs ([**4**] = 5×10^{-5} M) as a function of the equivalents of $Ce(NH_4)_2(NO_3)_6$ added at pH 8.0 (●, release of the first *p*-nitrophenolate; ○, release of the second *p*-nitrophenolate). [CHES buffer] = 5×10^{-3} M, [BNP] = 2×10^{-5} M, 25°C.

1) Manea, F.; Bindoli, C.; Polizzi, S.; Lay, L.; Scrimin, P. *Langmuir*, **2007**, *24*, *8*, 4120-4124.

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