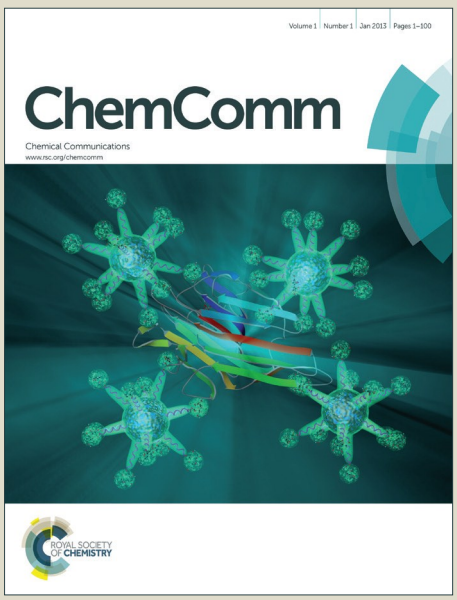
# ChemComm

Accepted Manuscript





ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

ARTICLE

# Supramolecular Phosphate Transfer Catalysis by Pillar[5]arene

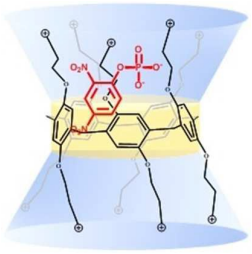
Daiane G. Liz[a] , Alex M. Manfredi[a], Michelle Medeiros [a], Rodrigo Montecinos[b], Borja Gómez-González[c], Luis Garcia-Rio*[c] and Faruk Nome*[a]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

A kinetic study on dinitrophenylphosphate monoester hydrolysis in the presence of a cationic pillararene, P5A, has been carried out. Formation of the supramolecular complex between phosphate ester and P5A has been studied by NMR showing complexation-induced upfield proton shifts indicative of aromatic ring inclusion in the pillararene cavity. Molecular dynamic calculations allow structure characterization for the 1:1 and 1:2 complexes. As a result of the supramolecular interaction both the acidity of DNPP and its hydrolysis rate constants are increased. Catalysis results from combination of both electrostatic stabilization reducing the negative electron density on the $PO_3^=$ oxygens and monoester dianion destabilization by the steric effects of close $NMe_3^+$ groups hindering the hydrogen-bonding with water and destabilising the monoester dianion.



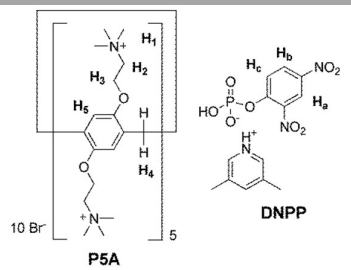
Supramolecular catalysis offers model systems with electrostatic and noncovalent interactions designed to mimic enzyme substrate interactions. Formation of host:guest complexes where the host carries a catalytic function can enhance reaction rates, with regioselectivity induced by spatial factors. Formation of host:guest complexes with a stoichiometry higher than 1:1 allows contact between two reagents in the restricted space of the host cavity, increasing local concentrations and so the rate of the reaction. Increased local concentration may be accompanied by desolvation of the reactants and stabilization of the transition state, thus yielding a true catalytic effect. In the present work we show, for the first time, that stabilization of the transition state results in a clear catalytic effect on phosphate transfer reactions in the presence of pillar[5]arenes. It is remarkable that phosphate transfer reactions[1-4] are fundamental for the chemistry of life despite the extreme stabilities of fully ionized mono- and dialkyl phosphate esters.

Pillar[n]arene macrocycle receptors, constructed from disubstituted hydroquinones linked by methylene bridges at the para position, were first reported in 2008[5]. New routes were rapidly developed to functionalize these receptors so that they exhibit host:guest properties that enable them to be employed in supramolecular systems such as chemosensors, drug delivery, and also in supramolecular aggregates[6-8]. Pillararenes can recognize guest molecules selectively in organic solvents, due in most cases to both an electron-rich cavity[9-11] and the formation of C-H···π interactions. But recognition also occurs, more interestingly, in aqueous media[12-16]. Very recently we reported[17] that a water soluble pillar[5]arene fully substituted with alkylammonium groups (P5A) forms very stable inclusion complexes with organic anions in competition with the macrocycle counterions. Based on this recognition ability we decided to study phosphate

[a] Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, SC, 88040-900 Brazil..
[b] Facultad de Química, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.
[c] Departamento de Química Física, Centro de Investigaciónen Química Biológica y Materiales Moleculares (CIQUS), Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain..

Electronic Supplementary Information (ESI) available: NMR titration of DNPP; kinetic rate analysis methodology, MD simulation for 1:2 complexes and experimental procedure. See DOI: 10.1039/x0xx00000x

transfer reactions to water-the medium in which biological chemistry takes place.




**Scheme 1.** Structures of the positively charged pillar[5]arene (P5A) and of the dianionic 2,4-dinitrophenylphosphate (DNPP).

Here we describe the use of a water soluble pillar[5]arene (P5A), which forms a supramolecular complex with the dianion 2,4-dinitrophenylphosphate (DNPP), and serves as a biomimetic model phosphatase, promoting the spontaneous hydrolysis of the phosphate monoester (structures shown in Scheme 1). The results are consistent with supramolecular complexation, which was studied in detail by molecular dynamics and spectroscopic techniques.

An NMR titration of 19.3 mM DNPP solution (Figure S-1) reveals a significant complexation-induced upfield shift. The upfield shifts of the DNPP signals are in the order Ha ≅ Hb >> Hc, the sequence indicating that the nitro group in position 4 is positioned deeper in the strongly shielding region of the P5A cavity. It is interesting to note that the P5A proton signals (H5, H4 and H2) show a small upfield shift (see Figure 1) and the protons H1 and H3 experience deshielding in the presence of DNPP, as evidenced by the downfield shift observed for these signals. Moreover the protons (H4, H3) tend to overlap. As previously reported[17] the up- and downfield shifts observed can be rationalized in terms of the exchange of the $Br^-$ counterions close to the functional groups of P5A and DNPP.

Left hand side of Figure 2 shows the starting point for the MD calculations of a system formed by 1 molecule of DNPP and 2 sodium ions incorporated into a pre-equilibrated system consisting of 1 molecule of pillar[5]arene and 10 bromide counterions solvated with 4200 SPC/E water molecules.

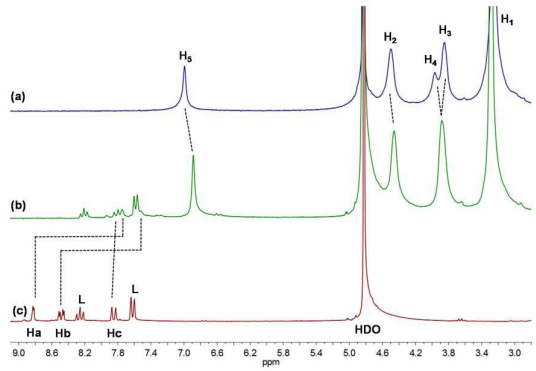



**Figure 1.** $^1$H NMR spectra ($D_2O$, 298 K, 200 MHz) (a) 17 mM P5A; (b) 17 mM P5A and 17 mM DNPP, (c) 17 mM DNPP. L correspond to the 2,6-lutidinium counter ion.

The model shows an initial electrostatic interaction between phosphate group and ammonium headgroup of P5A. The inclusion of the guest inside the cavity of the pillar[5]arene is observed after 1500 ps (Right hand side of Figure 2). The configuration of the supramolecular complex and the distance between P5A and DNPP does not change during the last 19 ns of calculation, consistent with the formation of the stable internal host-guest complex shown in the right hand side of Figure 2.

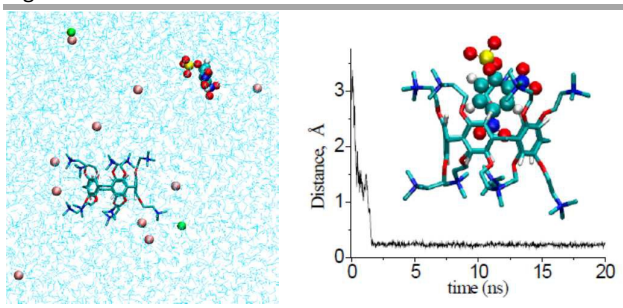



**Figure 2.** Left: The starting configuration, with DNPP in CPK representation and the pillar[5]arene in stick representation. Carbons are cyan, hydrogens white, nitrogens blue and oxygens (except for oxygen from water), red. Bromide and sodium are grey and green, respectively. For simplicity, water molecules are light blue in line representation. Right hand side shows a graph of the distance between the center of the P5A cavity and the carbon in position-4 of DNPP, as a function of calculation time and a snapshot of the 1:1 host-guest complex, after reaching equilibrium.

Experimental conditions with DNPP is in large excess over P5A allow the formation of a supramolecular complex between 2 molecules of DNPP and 1 molecule of pillar[5]arene. The model rapidly evolved to a structure where one DNPP molecule is maintained inside of the cavity, forming an internal complex. The second molecule is located on the opposite side of the cavity, interacting with the ammonium group and thus forming an external complex (Figure S-2).

The hydrolysis of DNPP was investigated in aqueous solutions at pH 7.0 (Bis-Tris buffer), where DNPP is present as the dianion[18] ( $pK_{a,w}$ =4.62) in both aqueous and supramolecular environments, at different concentrations of P5A. The rate vs. host concentration profile show that the rate constant increases with the concentration of P5A, reaching a plateau (Figure 3). A clear catalytic effect of P5A on the hydrolysis of DNPP is observed at pH=7. The observed rate constant increases almost ten-fold on increasing the pillararene concentration, reaching a limiting value for [P5A]>0.002M.

Based on NMR experiments and molecular dynamic calculations the catalytic effect should be ascribed to the formation of a 1:1 host:guest complex between P5A and DNPP and the hydrolysis reaction taking place simultaneously in both bulk water, $k_{D,w}$, and the cavity of pillararene, $k_{D,P5A}$. The observed rate constant is the sum of the rate constants in the two environments weighted by the molar fractions $X_{D,w}$ and $X_{D,P5A}$ of monoester dianion in each (see SI section).

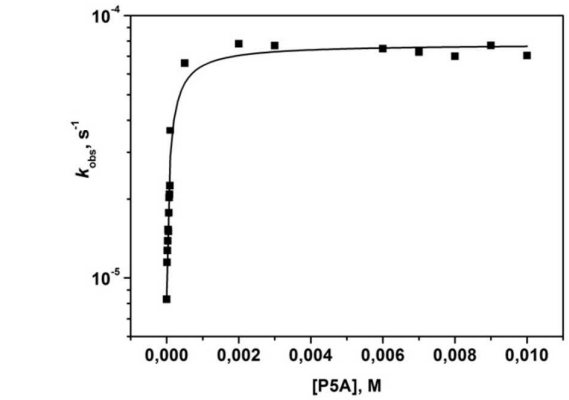



**Figure 3.** Influence of P5A concentration on the observed rate constant, $k_{obs}$, for DNPP hydrolysis, [DNPP]=5x10$^{-5}$M, in aqueous solutions at neutral pH, pH 7 (Bis-Tris buffer, and 25.0°C.

Analysis of kinetic data yield the binding constant for association of the dianion to pillar[5]arene, $K_{ass,D}$ = (5150 ± 660) $M^{-1}$, as well as the rate constant inside the cavity, $k_{D,P5A}$=(7.80±0.22)x10$^{-5}$ $s^{-1}$. This value is considerably higher than that observed in bulk water, $k_{D,w}$ =8.27 $10^{-6}$ $s^{-1}$ corresponding to a catalytic effect of almost ten-fold.

Complete characterization of the pillararene catalysis taking into account the acid/base equilibrium between the phosphate monoester dianion and monoanion was carried out by analyzing the pH effect on the DNPP hydrolysis rate constant both in the presence and absence of macrocycle. To this end observed rate constants have been obtained over the pH range 2-13 in the presence of 0.002 M P5A (in order to guarantee full DNPP incorporation into the pillararene) at 25.0ºC. The pH-rate profiles show that the rate constant increases with pH, as expected for the deprotonation of the monoanion and formation of the dianionic species of DNPP, reaching a plateau above pH 5, where DNPP is fully converted to the dianion. Figure 4 also compares the significant catalytic effect observed for the two reactions in the presence of P5A.

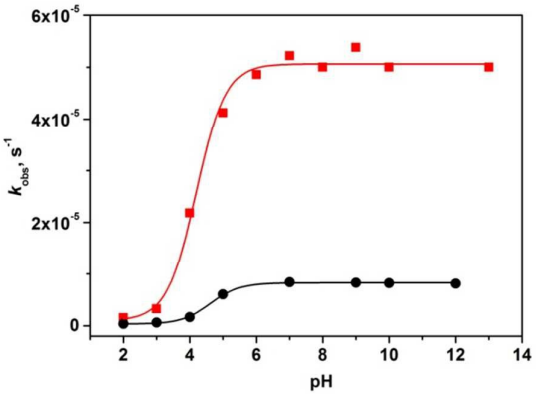
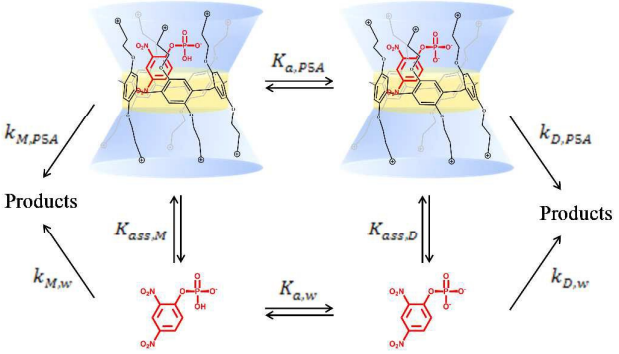



**Figure 4.** pH-Rate profiles for the hydrolysis of DNPP in the presence of pillar[5]arene (0.002 M) (■) and in aqueous solutions in the absence of pillar[5]arene (●), which were included for comparison purposes (data from reference 18).

The kinetic results are consistent with four simultaneous pathways: (*i*) monoanion in water, $k_{M,w}$), (*ii*) monoanion bound to the pillararene, $k_{M,P5A}$, (*iii*) dianion in water, $k_{D,w}$, and (*iv*) dianion bound in the pillararene cavity, $k_{D,P5A}$ (see Scheme 2 and SI section for a detailed description of data analysis).




**Scheme 2**

Equilibrium constant for dianion incorporation into P5A is 5.4-fold greater than that observed for the monoanion, as expected if electrostatic interaction is the main driving force for complexation. From Scheme 2 and data showed in Table 1 a value of $pK_{a,P5A}$=4.16±0.12 can be calculated for the acidity of DNPP monoanion inside the cavity of pillararene. Monoanion acidity increases by 3-fold on complexation as can be deduced by comparison of $pK_{a,P5A}$ and $pK_{a,w}$. This effect can be ascribed to the stronger binding of the dianion through electrostatic interaction with the positive charges of pillararene.

Main result from the present communication is the catalytic effect of pillararene on DNPP hydrolysis. The monoanion rate constant increases *ca*. 4-fold and that of the dianionic species almost 10-fold. The three equivalent "nonleaving group" oxygens of the $PO_3^{2-}$ group play important, well established roles in the reaction mechanism of monoester dianions. $O^{(-)}$ is also a potential electron donor, and given a very good leaving group OR* (Scheme 3) the triple nO–σ*P-OR* interactions available in all conformations of the dianion can lead to P–OR* cleavage under relatively mild conditions, with concurrent transfer of the $PO_3^-$ group to an available nucleophile.[19] For reactions in solution, the nucleophile must be present in the encounter complex but the reaction is primarily dissociative, driven by (and thus strongly dependent on) the stability of the leaving group. Accordingly, the reaction shows a very strong dependence on the pKa of the leaving group ($\beta_{LG}$=–1.0±0.2) and a small (but significant) effect on that of the nucleophile (βnuc=0.15±0.15).[2] The triple nO—σ*P–OR* electron-donation in the phosphate dianion represents the major driving force for displacement of the leaving group,[20] with the nucleophile behaving as acceptor in the phosphate transfer reaction: a consequence, of metaphosphate being too short-lived in water to exist as a solvent-equilibrated intermediate.[1-4]

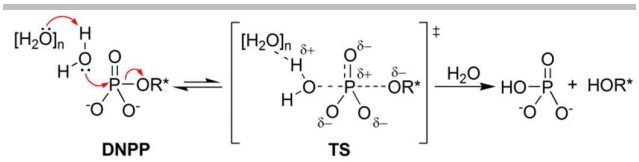
The proposed mechanism is fully consistent with the observation that enzyme-catalysed phosphate transfers

involving monoesters involve inversion of configuration.[2] Furthermore, fluoride and oxyanions show no readily measurable reaction at the phosphorus centers of 4-nitrophenyl or 2,4-dinitrophenyl phosphate dianions.[20,21]

**Table 1.** Values of the DNPP rate and equilibrium constants obtained from fitting equations 3-6 to experimental values showed in Figure 4.

| $K_{ass,M}$ / $M^{-1}$ | $K_{ass,D}$ / $M^{-1}$ | $k_{M,P5A}$ / $s^{-1}$ | $k_{D,P5A}$ / $s^{-1}$ |
|---|---|---|---|
| 774 ± 35 | (5150 ± 660) | $1.44x10^{-6}$ | $(7.80±0.22)x10^{-5}$ |
| $pK_{a,w}$ = 4.62; $k_{M,w}$ = $3.36x10^{-7}$ $s^{-1}$; and $k_{D,w}$ = $8.27x10^{-6}$ $s^{-1}$ (taken from ref 18) | | | |

Catalytic mechanism can be achieved by combination of $PO_3^=$ stabilization by electrostatic interaction and destabilization of the monoester dianion by disrupting the water structure. Electrostatic stabilization is compatible with the reported increase in rate constant for this reaction in water at high ionic strength[22] estimating a maximum 50-fold electrostatic effect on the rate constant. The nO—σ*P–OR* electron donation from the $PO_3^=$ group is significantly reduced by H-bonding solvation in water resulting in rate acceleration . Thus hydrolysis is accelerated in dipolar aprotic solvents. Electrostatic effects of the $NMe_3^+$ groups will reduce the negative electron density on the $PO_3^=$ oxygens and thus stabilise the dianion. But the steric effects of close $NMe_3^+$ groups will interfere with stabilising H-bonding to water and destabilise the monoester dianion.




**Scheme 3**

The major conclusion for this manuscript is that phosphate monoester hydrolysis is catalyzed inside the pillar[5]arene cavity. Catalysis results from combination of both: *(i)* electrostatic stabilization (fundamental for the incorporation of the dianion in the pillar[5]arene cavity) at the expense of reducing the negative electron density on the $PO_3^=$ oxygens and, *(ii)* steric effects of the wall of close $NMe_3^+$ groups surrounding the pillararene cavity, which interfere with stabilising H-bonding to water and, as a consequence, *destabilise* the monoester dianion speeding the reaction. Important repercussions can be deduced from the present study to biological chemistry by considering the relevance of phosphate transfer in biology. ATP in the human body is obtained from ADP and inorganic phosphate by a mechanism where the terminal bridge oxygen in ATP formed comes from ADP[23]. The simplest plausible mechanism involving inorganic phosphate as the phosphorylating agent demands a cationic general acid in the active site. Our results exploit the presence of electrostatic stabilization as a replacement for this general acid catalysis via the use of pillararenes as enzyme mimics.

**Acknowledgements**

We are grateful to INCT-Catálise/ CNPq, PRONEX, FAPESC, CSF/CNPq, CAPES, Ministerio de Economia y Competitividad of Spain (project CTQ2014-55208-P) and Xunta de Galicia (GRC2007/085). RM acknowledge financial support from project ICM-MINECOM, RC-130006-CILIS. Powered@NLHPC: This research was partially supported by the supercomputing infrastructure of the NLHPC (ECM-02)

**Notes and references**

1 A. J. Kirby, F. Nome, *Acc. Chem. Res.*, 2015, **48**, 1806-1814.
2 S. C. L. Kamerlin, P. K. Sharma, R. B. Prasad, A. Warshel, *Q. Rev. Biophys.*, 2013, **46**, 1-132.
3 J. K. Lassila, J. G. Zalatan, D. Herschlag, *Annu. Rev. Biochem.*, 2011, **80**, 669-702.
4 W. W. Cleland, A. C. Hengge, *Chem. Rev.*, 2006, **106**, 3252-3278.
5 T. Ogoshi, S. Kanai, S. Fujinami, T. Yamagishi, *J. Am. Chem. Soc.*, 2008, **130**, 5022-5023.
6 M. I. N. Xue, Y. Yang, X. Chi, Z. Zhang, F. Huang, *Acc. Chem. Res.*, 2012, **45**, 1294-1308.
7 P. J. Cragg, K. Sharma, *Chem. Soc. Rev.*, 2012, **41**, 597-607.
8 T. Ogoshi, T. Yamagishi, *Eur. J. Org. Chem.*, 2013, 2961-2975.
9 N. L. Strutt, R. S. Forgan, J. M. Spruell, Y. Y. Botros, J. F. Stoddart, *J. Am. Chem. Soc.*, 2011, **133**, 5668-5671.
10 C. Han, G. Yu, B. Zheng, F. Huang, *Org. Lett.*, 2012, **14**, 1712-1715.
11 N. L. Strutt, H. Zhang, M. A. Giesener, J. Lei, J. F. Stoddart, *Chem. Commun.*, 2012, **48**, 1647-1649.
12 T. Ogoshi, M. Hashizume, T. Yamagishi, Y. Nakamoto, *Chem. Commun.*, 2010, **46**, 3708-3710.
13 G. Yu, X. Zhou, Z. Zhang, C. Han, Z. Mao, C. Gao, F. Huang, *J. Am. Chem. Soc.*, 2012, **134**, 19489-19497.
14 C. Li, X. Shu, J. Li, S. Chen, K. Han, M. Xu, B. Hu, Y. Yu, X. Jia, *J. Org. Chem.*, 2011, **76**, 8458-8465.
15 X.-B. Hu, L. Chen, W. Si, Y. Yu, J.-L. Hou, *Chem. Commun.*, 2011, **47**, 4694-4696.
16 Y. Ma, X. Ji, F. Xiang, X. Chi, C. Han, J. He, Z. Abliz, W. Chen, F. Huang, *Chem. Commun.*, 2011, **47**, 12340-12342.
17 B. Gomez, V. Francisco, F. Fernandez-Nieto, L. Garcia-Rio, M. Martin-Pastor, M. R. Paleo, F. J. Sardina, *Chem. Eur. J.*, 2014, **20**, 12123-12132.
18 C. A. Bunton, E. J. Fendler, J. H. Fendler, *J. Am. Chem. Soc.*, 1967, **89**, 1221-1230.
19 E. A. Ruben, J. A. Plumley, M. S. Chapman, J. D. Evanseck, *J. Am. Chem. Soc.*, 2008, **130**, 3349-3358.
20 A. J. Kirby, A. G. Varvogli. *J. Chem. Soc. B*, 1968, 135-141.
21 A. J. Kirby, W. P. Jencks, *J. Am. Chem. Soc.*, 1965, **87**, 3209-3216.
22 D. Herschlag, W. P. Jencks, *J. Am. Chem. Soc.*, 1989, **111**, 7587-7596.
23 J. Weber, *Nat. Chem. Biol.*, 2010, **6**, 794–795.