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Communications

Binding of Phosphates to Abiotic Hosts in Water

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Summary: The foldable dicationic guanidinium host **2** binds biologically relevant phosphates in aqueous solution with K_a approaching 10^3 M^{-1}.

Inspired by the wide-spread occurrence of guanidinium anchor groups for oxoanions in natural receptors, numerous groups have incorporated this guanidinium moiety into abiotic hosts for phosphates.[1] Monotopic[2] and simple ditopic monoalkyl guanidinium compounds[3b] were shown to bind, e.g., HPO_4^{2-}, very weakly in water ($K_a < 16$ M^{-1}). However, some additional synthetic investment paid off in enhanced binding power[4] at least in media of low competition from protic solvents. Our pragmatic approach relies on the general concept of foldable r dular receptors[5] using chiral bicyclic guanidinium compounds as anchor groups. The conjunction of two of these binding modules resulted in a receptor capable to complex mononucleotides and some other anions in water.[6] We recently elaborated a similar ditopic host **1** which served to form 1:1 host–guest complexes with dicarboxylic anions in methanol.[7] However, the low solubility precluded the study of its binding features in water. The removal of the silyl protecting groups not only generated two additional hydroxy functions which might aid in guest binding but also rendered host **2** readily water soluble. Thus, the stage was set to characterize this artificial host as a receptor for biologically relevant phosphates in their natural environment.

The design of the foldable hosts feature two chiral bicyclic guanidinium units which are connected to a planar and rigid naphthalene spacer. Due to electrostatic repulsion of the positive charges these flexible receptors are supposed to adopt a variety of extended conformations. If there is any cooperative guest binding of the type observed in many enzymes[1] (i.e., the hydrogen bonding edge of the guanidinium anchor groups facing the oxygen atoms of a tetrahedral oxoanion as in **3**) the lateral wings must close in an hinge motion. By virtue of the chirality of the guanidinium moieties and the planarity of the naphthalene spacer the main planes of the anchor groups are oriented perpendicular to each other in an optima-larrangement to interact with the tetrahedral geometry of hydrogen bond acceptor sites of the guest. This *induced fit* binding process involves a drastic change in equilibrium conformation of the host correlating the "relaxed" unbound state with the much "tighter" and collapsed state in the host–guest complex. One must expect, of course, this folding to be energetically demanding and thereby counteracting guest binding. But at the same time the conformational change should be readily observable by NMR and might thus directly report on complex formation. This is an invaluable visualizing tool, since other

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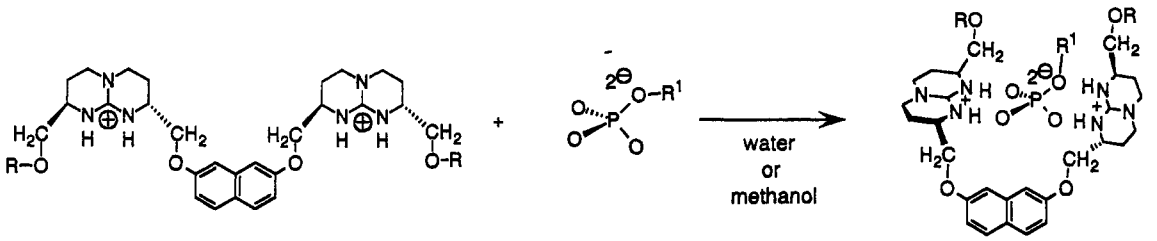
Scheme 1



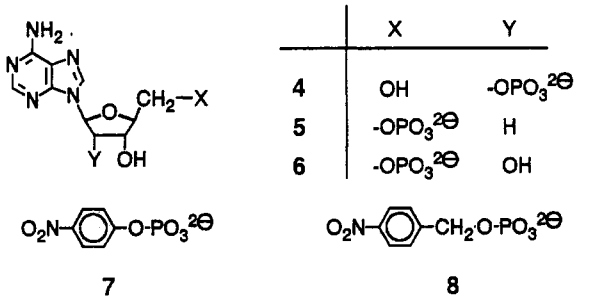
R	
1	$Si(Ph)_2{}^tC_4H_9$
2	H

3

Table 1. 1:1 Host–Guest Complexation of Phosphates by Host 1·2Cl⁻ in Methanol at Ambient Temperature As Determined by ^{1}H-NMR Titration and Nonlinear Curve Fitting[9]

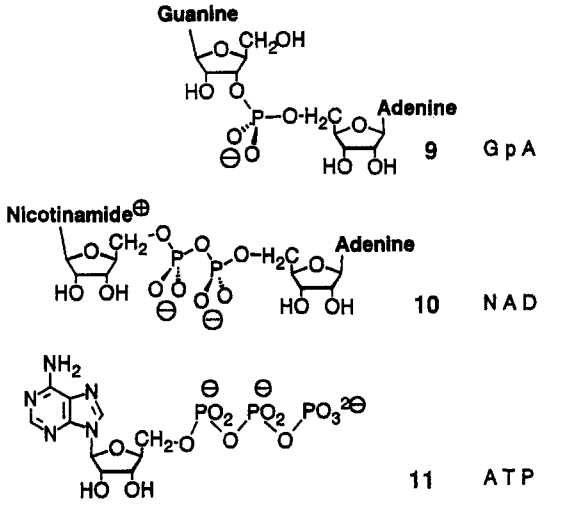
entry	phosphate	K_a (M^{-1})	$\Delta\delta_{max}$[d] (Hz)
1	HPO_4^{2-} [a,b]	18 300	26.9
2	2′-AMP^{2-} (4)[c]	18 200	46.9
3	2′-AMP^{2-} (4) in DMSO[a]	5250	52.5
4	2′-dAMP^{2-} (5)[c]	29 200	36.4
5	5′-AMP^{2-} (6)[c]	38 000	32.8

[a] 1·2ClO_4^-. [b] [K^+[18]crown-6] salt. [c] 2-(*tert*-Butylimino)-2-(diethylamino)-1,3-dimethylperhydrodiazaphosphorin (BEMP; Fluka) salt. [d] At 250 MHz.



	X	Y
4	OH	$-OPO_3^{2\ominus}$
5	$-OPO_3^{2\ominus}$	H
6	$-OPO_3^{2\ominus}$	OH

7 8



9 GpA

10 NAD

11 ATP

physico-chemical properties including the ^{31}P-chemical shift of phosphates are notoriously insensitive to host–guest binding.[8]

The respective ^{1}H-NMR titration experiments of phosphates with the dicationic host 1 in methanol could easily be fitted to a 1:1 host–guest binding stoichiometry.[9] The association constants collected in Table 1 show that phosphate guests can be complexed with considerable strength in hydrogen-bonding solvents. Compared to hydrogen phosphate the ester with a secondary alcohol (2′-AMP (4)) possesses an identical association constant. Switching the solvent to straight DMSO cuts complex stability to one third, reflecting the dominance of electrostatic binding interactions. The higher dielectric constant [ϵ (DMSO) = 46.7; ϵ (CH_3OH) = 32.6] and stronger donicity [donor strength DS (DMSO) = 27; DS (CH_3OH) = 16][10] make DMSO the more effective competitor for the hydrogen bonding sites of the host.

If the anionic moiety is connected to a primary alcoholic carbon and thus more easily accessible, the complex stability is enhanced by more than 2-fold. But more subtle structural differences in the guest may modulate binding strength as well. The extra OH group allows 6 to form a stronger host–guest complex than 5 suggesting the participation of an additional hydrogen bond.

Similar ^{1}H- and ^{31}P-NMR titration studies with host 2 in methanol showed odd behavior indicative of complexes of higher stoichiometries. Apparently, this host lacking the bulky silyl-protecting groups possesses more and alternative interaction modes with phosphate guests in this solvent which thwarted our attempts to extract meaningful association constants.

Under the most stringent solvation conditions that an oxoanionic substrate can face, i.e., in aqueous solution, the freely soluble host 2 cleanly forms 1:1 complexes with the majority of organic phosphate guests. The results of our binding studies are collected in Table 2 and show a distinctly different selectivity pattern compared to the binding features of the analogous host 1 in methanol. Amazingly, inorganic phosphate is bound substantially better than phosphate esters of the same charge. We expected the organic moieties to assist in binding by hydrogen and/or hydrophobic bonding, but they rather appear to break up the favorable noncovalent bonding network established between HPO_4^{2-} and 2 which is evidenced by the high association constant. This parallels the results of Springs and Haake[2] with simple guanidinium ion and contrasts those obtained with polyammonium

(8) *Phosphorus-31 NMR*; Gorenstein, D. G., Ed.; Academic Press: New York, 1984; p 31 ff. For successful applications see, however, refs 1 and 4.

(9) The titration curves were analyzed by nonlinear regression fitting to a 1:1 binding model; software: ENZFITTER, Biosoft, Cambrigde, UK.

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Table 2. 1:1 Host–Guest Binding of 2 with Phosphates in Water As Determined by ^{1}H-NMR Titration at Ambient Temperature[9]

entry	phosphate	K_a (M^{-1})	$\Delta\delta_{max}$[c] (Hz)
6	HPO_4^{2-} [b]	970	11.6 ± 0.2
7	5′-AMP^{2-} (6)[a]	204	67.6
8	5′-AMP^{2-} (6) in MeOH[a]	9330	31.3
9	*p*-nitrophenyl phosphate^{2-} (7)[a]	105	31.0
10	*p*-nitrobenzyl phosphate^{2-} (8)[a]	530	60.2
11	guanylyl(3′–5′)adenosine (GpA)$^{1-}$ (9)[a]	54	26.4
12	nicotinamide–adenine dinucleotide (NAD)$^{1-}$ (10)[a]	140	130
13	ATP^{4-} (11)[a]	840	65.4

[a] 2·2Br$^-$. [b] 2·2Cl$^-$. [c] At 250 MHz.

receptors.[11c] The complexation of 5′-AMP^{2-} (6) in water is considerably weaker (factor >40) than in methanol, which again underlines the dominance of electrostatic over solvophobic binding interactions. It was expected to see increased host–guest complexation with ATP^{4-} (entry 13) due to its higher charge, but 2 must be regarded as a mediocre host for ATP^{4-} since better[11c] as well as much less efficient receptors[11a,12] have been decribed. In spite of the flexibility of host 2 certain guests are only poorly accommodated. *p*-Nitrophenyl phosphate (7) was originally supposed to provide a hydrophobic moiety capable of interaction with the aromatic naphthalene spacer unit of 2. The small association constant suggests that there is no easy way to satisfy this multiple interaction mode. If the strain is somewhat released by the introduction of an additional methylene carbon between the ionic and hydrophobic substructures of the guest 8 we observe a 5-fold greater complex stability (entry 10). On the other side the very hydrophilic sugar phosphates (e.g., glucose 6-phosphate) do not serve as host–guest binding partners for 2 in water at all.

De Mendoza et al.[13] were able to extract monoanionic phosphate diesters by specially designed guanidinium salts from an aqueous solution into chloroform. Though this does not prove the existence of significant amounts of the corresponding host–guest complexes in water there is a fair chance that even with monoanionic phosphate species host–guest binding can be detected with suitable artificial hosts. The respective experiment (entry 11) identified 2 as a low-affinity host (K_a = 54 M^{-1}) for the GpA nucleotide 9 in homogeneous aqueous solution and opens the perspective for sequence selective binding of related abiotic receptors to nucleic acids. It did not come as a surprise to see better binding of the coenzyme NAD 10 to 2 because its site of interaction most likely is the dianionic diphosphate diester moiety, the charge of which is partly compensated by the pyridinium heterocycle. Interestingly, this internal diphosphate of NAD is anchored to the guanidinium side chain of arginine in a number of dehydrogenase enzymes.[14]

In conclusion, we have shown that low charged but suitably designed artificial receptors like 2 can indeed form reasonably stable host–guest complexes with biologically relevant phosphates in their natural environment.

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Supplementary Material Available: Experimental procedure and characterization data for 2 and figures of NMR titrations (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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