Table III. Flux of Potassium Perchlorate for Functionalized Dibenzo-18-crown-6

carrier	$10^8 J$,[a] mol cm^{-2} h^{-1}
1	24.9 ± 1.4
2	20.4 ± 0.6
3	8.3 ± 0.9

[a] $[KClO_4]^\circ_{sp} = 10^{-1}$ M; $[\overline{carrier}]^\circ = 10^{-2}$ M; Accurel support; T = 298 K.

partition coefficient of the crown ether between organic and aqueous phase greater than 10^5. The flux of potassium perchlorate across the membrane was measured for each of the crown ethers and the data are listed in Table III. The introduction of two acyl substituents lowers the flux despite a higher effective crown ether concentration in the membrane phase. Because the transport is determined by diffusion through the membrane phase (vide supra) the diminution of the flux is caused substantially by a lower value of the diffusion coefficient of **2**.[26] The difference in flux between **2** and **3** is great, while the difference in structure is small. The acyl substituent attached to the aryl ring diminishes the partition coefficient in contrast with the alkyl substituent,[28] resulting in a better interaction with the water molecules at the interface as well as a higher complex concentration. Therefore, we suggest that when the lipophilic character of the carrier is too pronounced, the transport is no longer limited by diffusion through the membrane phase but by complex formation at the interface between the source phase/membrane phase.

Conclusions

For the system investigated in this study the transport of potassium perchlorate through the membrane is determined by the diffusion of the complex through the membrane phase. In the membrane phase the cation complex and the anion are present as free ions, which was ascribed to the high value of the dielectric constant. The values of the effective diffusion coefficient D_0 and the complexed fraction of the crown ether are $(3.2 \pm 0.5) \times 10^{-7}$ cm^2 s^{-1} and 0.35 ± 0.07, respectively. The diffusion of the crown ether through the membrane phase is dependent on the support and is influenced only by the porosity of the membrane for Accurel or by the porosity as well as the tortuosity of the membrane for Celgard 2500. The partition coefficient of the carrier is very important in these systems; the effective concentration of **1** in the membrane phase is reduced by 40 mol % of the initial crown ether concentration by dissolution in the aqueous phases.

Registry No. 1, 14187-32-7; $KClO_4$, 7778-74-7; Accurel, 9002-88-4; Celgard 2500, 9003-07-0; polypropylene, 9003-07-0; *o*-nitrophenyl octyl ether, 37682-29-4.

(26) Besides the diffusion coefficient also the complexation constant will influence the flux. Ungaro[27] has measured substituent effects on the stability of cation complexes of 4′-substituted monobenzo crown ethers in acetone. He found that the stabilities of complexes of Na^+ with benzo-15-crown-5 (log $K(Na^+)$ = 3.54) and of K^+ with benzo-18-crown-6 (log $K(K^+)$ = 5.10), respectively, decreased with a formyl substituent (4′-formylbenzo-15-crown-5, log $K(Na^+)$ = 3.05; 4′-formylbenzo-18-crown-6, log $K(K^+)$ = 4.89). A lower value of the complexation constant is related to a lower flux, but the effect is too small for a complete explanation.

(27) Ungaro, R.; El Haj, B.; Smid, J. *J. Am. Chem. Soc.* **1976**, *98*, 5198.

(28) The effect of the carbonyl substituent on the partition coefficient can be demonstrated by comparison of ethylbenzene with acetophenone for the system 1-octanol/water:[22] log P(ethylbenzene) = 3.13 and log P(acetophenone) = 1.70.

Supramolecular Catalysis of Phosphoryl Transfer: Pyrophosphate Synthesis from Acetyl Phosphate Mediated by Macrocyclic Polyamines†

Mir Wais Hosseini and Jean-Marie Lehn*

Contribution from the Institut Le Bel, Université Louis Pasteur, 67000 Strasbourg, France.
Received March 27, 1987

Abstract: The macrocyclic polyamine [24]-N_6O_2 (**1**) catalyzes the hydrolysis of acetyl phosphate (AcP) to orthophosphate (P) and the synthesis of pyrophosphate (PP) from an intermediate **1**-PN and P. The course of these reactions was monitored by ^{31}P NMR spectroscopy, which revealed the formation of PP and of an intermediate which was identified as the monophosphorylated macrocycle **1**-PN by ^{1}H and ^{31}P NMR studies. The rate of **1**-PN disappearance was first order and decreased with increasing pH of the solution. The first-order rate constants on AcP consumption showed a maximum at pH 7 with an acceleration factor of about 5 at 40 °C. In the presence of an excess of compound **1**, a rate saturation was observed at pH 7. With an excess of AcP, compound **1** reacted until all AcP was consumed, indicating that the process was catalytic. Addition of competitive anions such as AMP^{2-}, SO_4^{2-}, and HPO_4^{2-} retarded both AcP consumption and **1**-PN formation and decreased the yield of PP, except for P, which increased markedly the amount of PP generated. The formation of PP probably involves precomplexation of P by the **1**-PN intermediate followed by an intramolecular phosphoryl transfer from PN to P. The mechanism of PP synthesis was analyzed by ^{18}O-labeling, inhibiton, and saturation experiments. The ditopic receptor molecule **1** performs a process of *cocatalysis* in which its two subunits cooperate for bringing together reagents and inducing bond formation. It provides at the molecular level conditions suitable for PP synthesis by binding AcP and mediating two phosphoryl transfers from AcP to **1**, giving **1**-PN, and then from **1**-PN to P. Studies of 11 related macrocyclic polyamines **2**–**12** allow analysis of structural effects on the rates and products of the AcP reaction. They indicate that both amine basicity and geometry effects operate; PN formation is more influenced by the former, whereas PP generation depends on both and is much more sensitive to structural variations.

Reactivity and catalysis represent major features of the functional properties of supramolecular systems.[1-4] Molecular receptors bearing appropriate functional groups may bind selectively a substrate, react with it, and release the products. Supramolecular reactivity and catalysis thus involve two main steps: *recognition* of the substrate, followed by *transformation* of the bound species into products. The design of efficient and selective molecular catalysts may give mechanistic insight into the elementary steps

† Dedicated to the memory of Professor Iwao Tabushi.

(1) (a) Lehn, J. M. *Science (Washington, D.C.)* **1985**, *227*, 849–856. (b) Lehn, J. M. *Pure Appl. Chem.* **1978**, *50*, 871–892.

(2) Lehn, J. M. *Pure Appl. Chem.* **1979**, *51*, 979–997. (b) Lehn, J. M. In *Int. Symp. Bioorg. Chem.* (Brelow, R., Ed.) *Ann. N.Y. Acad. Sci.* **1986**, *471*, 41–50.

0002-7863/87/1509-7047$01.50/0

of catalysis, provide new types of chemical reagents, and produce models of reactions effected by enzymes that reveal factors contributing to enzymatic catalysis.[1-6]

Bond-cleavage reactions have been extensively studied in this respect. A further step lies in the design of systems capable of inducing *bond formation*, which would thus effect *synthetic* reactions as compared to degradative ones. In order to realize "bond-making" rather than "bond-breaking" processes, the presence of several binding and reactive groups is essential. Such is the case for coreceptor molecules in which subunits may cooperate for binding and transformation of the substrates;[3] as a consequence, they should be able to perform *cocatalysis* by bringing together substrate(s) and cofactor(s) and mediating reactions between them within the supramolecular complex.[1a,2b,3]

In view of the important role played by anions in chemical as well as biological processes, the binding of organic and inorganic anions by organic ligands would be expected to provide a multitude of novel structures with properties of wide significance. The development of the chemistry of anion complexation by macrocyclic polyammonium receptor molecules (ref 1 and 7-12 and references therein) opens the way to the design of molecular catalysts capable of effecting reactions on bound anionic substrate(s).

Phosphoryl transfer processes are of special interest in this regard, since they usually involve anionic species and can take place in bond-formation as well as bond-cleavage reactions; furthermore, they play a fundamental role in the energetics of all living organisms.[13-15]

We have shown earlier that macrocyclic polyamines, in particular the [24]-N_6O_2 macrocycle **1**, catalyze ATP, ADP, and pyrophosphate hydrolysis.[16,17] The effect of metal cations on this reaction has also been studied.[18] In the course of ATP hydrolysis catalyzed by compound **1**, an intermediate, which was tentatively identified as a phosphoramidate (PN) derivative of **1**, was observed.[16,17] In order to establish the structure of this intermediate, it was necessary to isolate larger amounts of it. To this end, the hydrolysis of the phosphorylating agents acetyl phosphate (AcP = $CH_3COOPO_3^{2-}$), creatine phosphate, arginine phosphate, and phosphoenol pyruvate in the presence of **1** was investigated. Among these substrates, only in the case of AcP could the PN intermediate be obtained; in addition, inorganic pyrophosphate (PP) was generated via a process which was proposed to involve

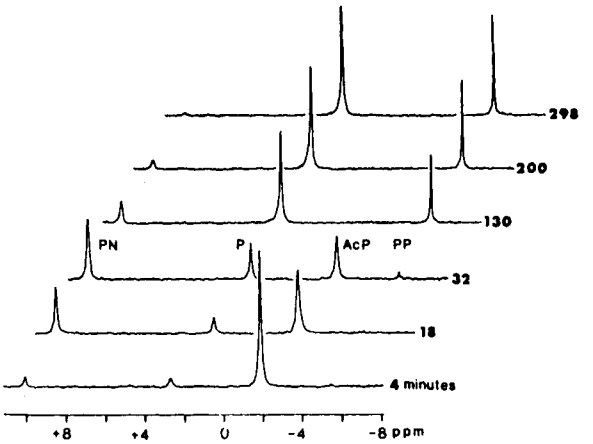
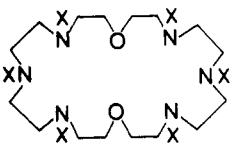
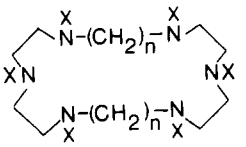


Figure 1. Observation of the reaction of acetyl phosphate (AcP) in the presence of macrocycle **1** by ^{31}P NMR spectroscopy at 81 MHz (with proton decoupling) as a function of time (min) (in D_2O/H_2O 1/9 solution containing initially 0.03 M AcP and 0.03 M **1** at pH 7, 40 °C (PN = phosphoramidate (**1**-PN), P = inorganic phosphate, PP = inorganic pyrophosphate). The chemical shifts are given with respect to external 85% H_3PO_4.

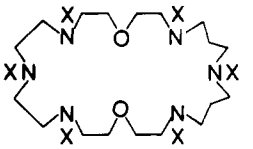
Chart I



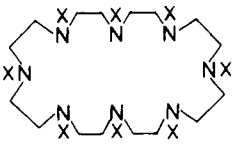
1, X = H
2, X = Me
3, X = $CH_2CH_2NH_2$
3a, X = CH_2CH_2NHTs



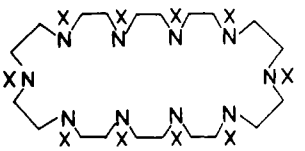
5, X = H; n = 2
6, X = H; n = 4
6a, X = Ts; n = 4
7, X = H; n = 5
7a, X = Ts; n = 5
8, X = H; n = 9



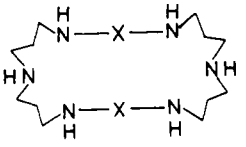
4, X = H
4a, X = Ts



9, X = H



10, X = H
10a, X = Ts



11, X = $CH_2CH_2CH_2$
12, X = $(CH_2CH_2CH_2)_2NH$

$XN(CH_2CH_2NXY)_2$

13a, X = Ts; Y = H
13b, X = Ts; Y = $CH_2CH_2OCH_2CH_2OH$
13c, X = Ts; Y = $CH_2CH_2OCH_2CH_2OMs$
13d, $TsN(CH_2CH_2CH_2NHTs)_2$

$XN(CH_2CH_2NXCH_2CH_2NXY)_2$

14a, X = Ts; Y = H
14b, X = Ts; Y = CH_2CH_2OH
14c, X = Ts; Y = CH_2CH_2OMs

reaction of the phosphorylated intermediate with phosphate (P).[19]

We now report a detailed study of this *pyrophosphate synthesis* mediated by macrocycle **1**, together with a broader investigation concerning the effect of a wide range of macrocyclic polyamines,

(3) Lehn, J. M. In *Biomimetic Chemistry*; Yoshida, Z. I., Ise, N., Eds.; Elsevier: New York, 1983; pp 163-187.
(4) Sirlin, C. *Bull. Soc. Chim. Fr.* **1984**, 15-40.
(5) Jencks, W. P. In *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969.
(6) (a) Breslow, R. *Science (Washington, D.C.)* **1982**, *218*, 532-537. (b) Kellogg, R. M. *Top. Curr. Chem.* **1982**, *101*, 111-145. (c) Tabushi, I.; Yamamura, K. *Ibid.* **1983**, *113*, 145-182. (d) Murakami, Y. *Ibid.* **1983**, *115*, 107-155. (e) Kellogg, R. M. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 782-794.
(7) Dietrich, B.; Hosseini, M. W.; Lehn, J. M.; Sessions, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 1282-1283. Hosseini, M. W.; Lehn, J. M. *Helv. Chim. Acta* **1987**, *76*, 1312-1319.
(8) Kimura, E.; Kodama, M.; Yatsunami, T. *J. Am. Chem. Soc.* **1982**, *104*, 3182-3187.
(9) (a) Hosseini, M. W.; Lehn, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 3525-3527. (b) Hosseini, M. W.; Lehn, J. M. *Helv. Chim. Acta* **1986**, *69*, 587-603.
(10) Gelb, R. I.; Lee, B. T.; Zompa, L. J. *J. Am. Chem. Soc.* **1985**, *107*, 909-916.
(11) Vögtle, F.; Sieger, H.; Müller, W. M. *Top. Curr. Chem.* **1981**, *98*, 143-161.
(12) Pierre, J. L.; Baret, P. *Bull. Soc. Chim. Fr.* **1983**, 367-379.
(13) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877-919.
(14) Ramirez, F.; Marecek, J. F. *Pure Appl. Chem.* **1980**, *52*, 1021-1045.
(15) Rösch, P. *Prog. NMR Spectrosc.* **1986**, *18*, 123-169.
(16) Hosseini, M. W.; Lehn, J. M.; Mertes, M. P. *Helv. Chim. Acta* **1983**, *66*, 2454-2466; **1985**, *68*, 818.
(17) (a) Hosseini, M. W.; Lehn, J. M.; Maggiora, L.; Mertes, K. B.; Mertes, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 537-544. (b) Blackburn, G. M.; Thatcher, G. R. J.; Hosseini, M. W.; Lehn, J. M. *Tetrahedron Lett.* **1987**, 2779-2782.
(18) Yohannes, P. G.; Mertes, M. P.; Mertes, K. B. *J. Am. Chem. Soc.* **1985**, *107*, 8288-8289. Yohannes, P. G.; Plute, K. E.; Mertes, M. P.; Bowman-Mertes, K. *Inorg. Chem.* **1987**, *26*, 1751-1755.
(19) Hosseini, M. W.; Lehn, J. M. *J. Chem. Soc., Chem. Commun.* **1985**, 1155-1157.

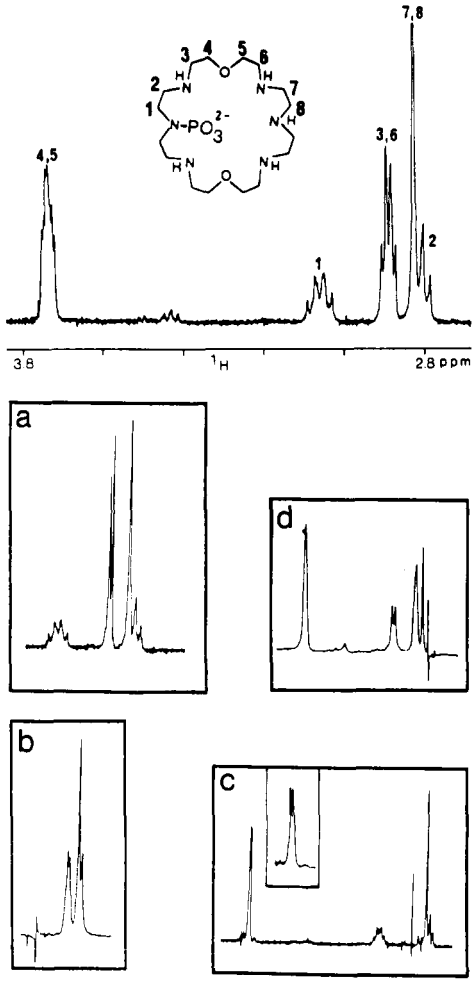


Figure 2. 400-MHz ^{1}H NMR spectrum of the phosphoramidate intermediate **1**-PN (top) and homonuclear ^{1}H{^{1}H} decoupling experiments (pH ~14, in D_2O). Decoupled spectra (bottom) correspond to irradiation of CH_2 protons (a) 4, and 5, (b) 1, (c) 3 and 6, and (d) 2. The chemical shifts are given with respect to internal (trimethylsilyl)-propylenesulfonate; the signals around 3.4 ppm are due to solvents used in the extraction procedure.

compounds **2**–**12** (Chart I), on the same reaction. The results obtained provide a basis for analyzing the mechanism and the structural requirements of the PP formation process.

Results

Observation of the Reaction of Macrocyclic Polyamines 1–12 with Acetyl Phosphate. The reaction of AcP in the presence or absence of macrocyclic polyamines was followed by ^{31}P and ^{1}H NMR spectroscopy in aqueous solution at different pH values. Whereas AcP hydrolyzed in water to phosphate (P) and acetate, two additional species were observed in the presence of compound **1** at pH 7: pyrophosphate (PP) and a transient intermediate (PN) giving a ^{31}P NMR signal at ca. 10 ppm, which first accumulated and then disappeared (Figure 1). PP itself slowly gave P.[16,17] These PP and PN signals were never detected (<2%) in the absence of **1** under the conditions used here. Furthermore, after all AcP had reacted, the PP signal still increased in intensity while PN decreased, indicating that PP formation was linked to a reaction involving PN.

Structure of the PN Intermediate Derived from Macrocycle 1. The nature of the PN intermediate is of key importance for understanding the present processes as well as ATP hydrolysis in presence of **1**.[16,17] After the intermediate was separated from the other compounds present in the solution (see Experimental Section), its structure could be established as **1**-PN by detailed analysis of its ^{1}H and ^{31}P spectra. The ^{1}H NMR signals were assigned by a series of ^{1}H{^{1}H} and ^{31}P{^{1}H} decoupling experiments (Figure 2): CH_2O, two triplets (4, 5) (J_{HH} = 6 Hz); NCH_2CH_2O,

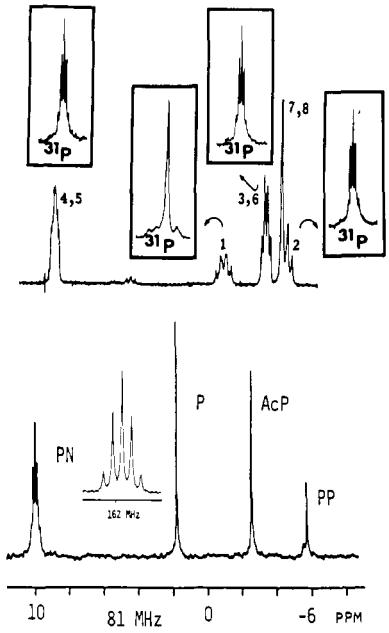


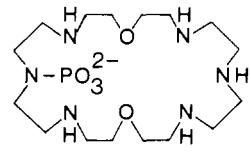
Figure 3. Heteronuclear ^{31}P{^{1}H} decoupling experiments. Undecoupled 400-MHz ^{1}H (top) and 81-MHz ^{31}P (bottom) NMR spectra of the intermediate **1**-PN; the ^{31}P spectrum has been measured on the reaction mixture containing also P, AcP, and PP. The inserts (top) represent the ^{31}P NMR signal of **1**-PN under irradiation of the proton signal indicated; the insert (bottom) shows an expansion of the ^{31}P quantuplet measured at 162 MHz.

two triplets (3, 6) (J_{HH} = 6 Hz); CH_2NP, quartet (1) (J_{HH} = 9.7 Hz; J_{PH} ~ 9.5 Hz); NCH_2CH_2NP, triplet (2) (J_{HH} = 7.3 Hz); NCH_2CH_2N, single (7, 8). The downfield position of the ^{31}P NMR signal (~+10 ppm) and its quintuplet structure (J_{PH} = 9.5 Hz; Figure 3), resulting from coupling to two equivalent CH_2 groups, indicated that PN must be an *N*-phosphoryl derivative of macrocycle **1**. The proton spectrum showed a 2:1 total intensity ratio for CH_2O (4, 5) or CH_2CH_2O (3, 6) groups with respect to CH_2NP (1). Observation of two triplets for both CH_2O (4, 5) and CH_2CH_2O (3, 6) indicated a left-to-right dissymmetry in the PN compound.

Homonuclear ^{1}H{^{1}H} Decoupling Experiments (Figure 2). Selective decoupling of signal (4, 5) in the spectrum of **1**-PN did not affect (2) and (7, 8) but transformed the two triplets (3) and (6) into two singlets. On the other hand, when (3) and (6) were irradiated, two singlets were obtained for (4) and (5) and the other signals were not altered. Selective decoupling of (1) changed (2) into a singlet without affecting other signals and finally, selective decoupling of (2) gave a doublet for (1) with a coupling constant of 9.5 Hz.

Heteronuclear ^{31}P{^{1}H} Decoupling Experiments (Figure 3). Observation of the ^{31}P signal of **1**-PN while protons were selectively irradiated allowed assignment of the connectivity between protons and phosphorus. Indeed, selective irradiation of the (4, 5), (3, 6), (7, 8), and (2) signals did not change the quintuplet structure observed by ^{31}P NMR; only irradiation of proton signal (1) transformed the quintuplet structure into a singlet.

The ^{1}H and ^{31}P NMR spectra together with the homo- and heteronuclear decoupling experiments allowed us to unambiguously assign to the PN intermediate the monophosphoramidate structure **1**-PN, resulting from selective phosphorylation of the central nitrogen site of a single diethylenetriamine subunit of macrocycle **1**.



1-PN

Table I. Effects of pH on the Observed First-Order Rate Constants ($k_{obsd} \times 10^3$ min^{-1})[a] for Disappearance of Acetyl Phosphate (AcP) (30 mM) and on the Maximum Amounts of **1**-PN and PP Formed in the Absence or in the Presence of Macrocyclic Polyamine **1** (30 mM) in H_2O/D_2O 9/1 at pH 7 and 84 °C or 40 °C[b]

	pH						
	3	5	6	7	8	9	11
k_{obsd} in the absence of **1**	176 (17.3)	112		107 (7.0)	116	(6.3)	(6.1)
k_{obsd} in the presence of **1**	135 (3.5)	276	437	840 (36.0)	475	400 (11.6)	(7.46)
% **1**-PN_{max}[c]	0 (0)	15	32	40 (43)	56	(42)	(0)
% PP_{max}[d]	8 (6)	22	25	26 (35)	24	(0)	(0)

[a] k_{obsd} ±10% at 40 °C, ±20% at 84 °C. [b] Values in parentheses at 40 °C. [c] Percent of **1**-PN in total P when all AcP is consumed. k_{obsd} (disappearance of PN) $\times 10^3$ min^{-1} at 84 °C: 362 at pH 6, 280 at pH 7, 162 at pH 8; at 40 °C: 13.0 at pH 7, very slow at pH 9 (<2% decomposition in 5 h). [d] Percent of PP given in P content (see Experimental Section) when all the intermediate **1**-PN in consumed.

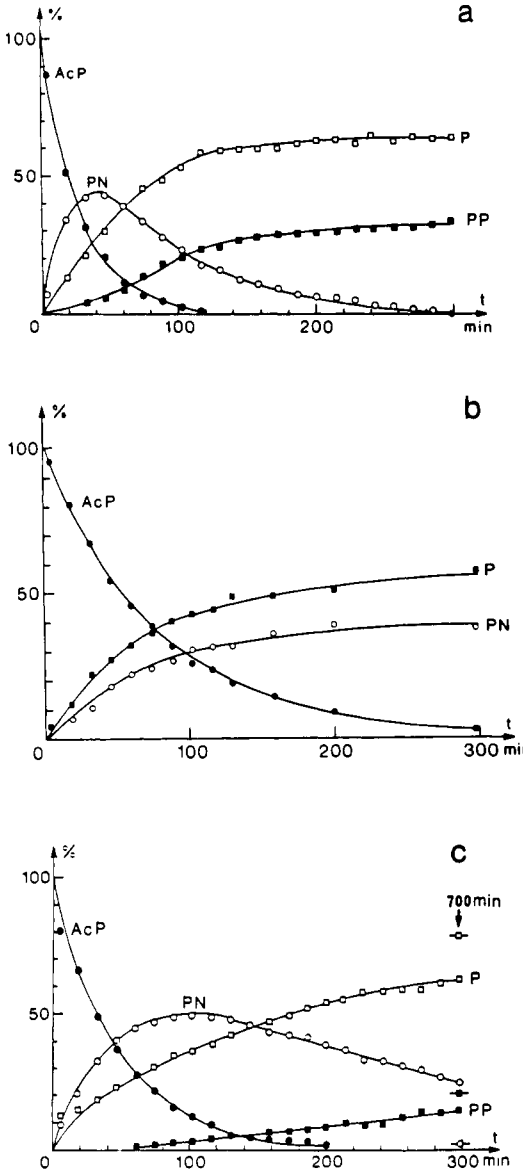


Figure 4. Time dependence of the reaction components of AcP hydrolysis catalyzed by macrocycle **1**. Plot of the observed percentages of AcP, **1**-PN, PP, and P vs. time (min) in D_2/H_2O 1/9 solution containing initially 0.03 M AcP and 0.03 M **1** at 40 °C: (a) at pH 7; (b) at pH 9; (c) at pH 7 in the presence of 2 equiv of AMP (the percentage toward the end of the reaction (700 min) is also indicated). % PP are given in P content (see Experimental Section).

Kinetic and Mechanistic Results on the AcP Reaction. The evolution of the proportion of the various species (AcP, P, PP) observed by ^{31}P NMR spectroscopy during the reaction of AcP in the presence of 1 equiv of macrocycle **1** is shown in Figure 4a,b for pH 7 and pH 9 at 40 °C. The corresponding plots of AcP consumption and of **1**-PN disappearance (after all AcP had been consumed) as a function of time (pH 7; Figure 5) gave apparent

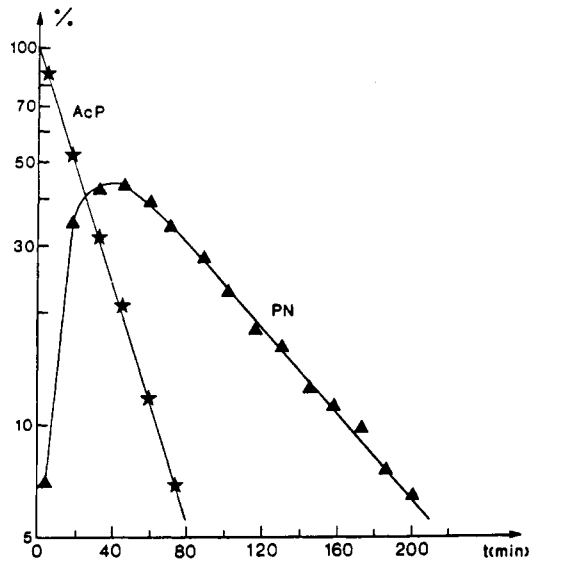


Figure 5. Rates of AcP and **1**-PN disappearance. Semilogarithmic plots of the percentage of remaining AcP and **1**-PN vs. time (min) in D_2O/H_2O 1/9 solution containing initially 0.03 M AcP and 0.03 M compound **1** at 40 °C and pH 7.

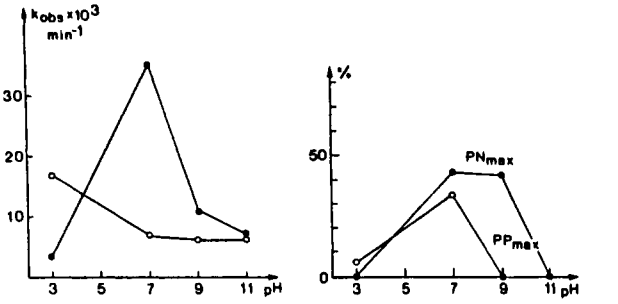


Figure 6. pH dependence of the rate of AcP hydrolysis uncatalyzed and catalyzed by macrocycle **1** (left) and pH dependence of the maximum percentage of **1**-PN and PP (right). Plot orf $k_{obsd} \times 10^3$ min^{-1} vs. pH for (●) hydrolysis of 0.03 M AcP and macrocycle **1** at 40 °C in D_2O/H_2O 1/9 and (○) uncatalyzed rate of hydrolysis of 0.03 M AcP at 40 °C (left). Plot of **1**-PN_{max} (●) and PP_{max} (○) vs. pH at 40 °C (right). % PP are given in P content (see Experimental Section).

first-order rate constants of $k_{obsd} = 0.036$ min^{-1} for AcP and of $k_{obsd} = 0.013$ min^{-1} for **1**-PN. Under the same conditions, but with 2 equiv of compound **1**, the values $k_{obsd} = 0.041$ and 0.011 min^{-1} were obtained for AcP and **1**-PN, respectively. These values are very close to those obtained with 1 equiv of **1**, indicating rate saturation.

The pH of the solution affected strongly the observed rate of AcP and of **1**-PN disappearance and the amount of **1**-PN and of PP formed at a given reaction time (Table I; Figure 6); the highest rate constant and largest amount of PP were obtained at pH 7; at pH 3, 6–8% PP was produced, although the **1**-PN intermediate was not detected; at pH 11, neither **1**-PN nor PP was observed (for the definition of % PP, see Experimental Section). The rate of **1**-PN disappearance decreased from pH 6 to 9. AcP

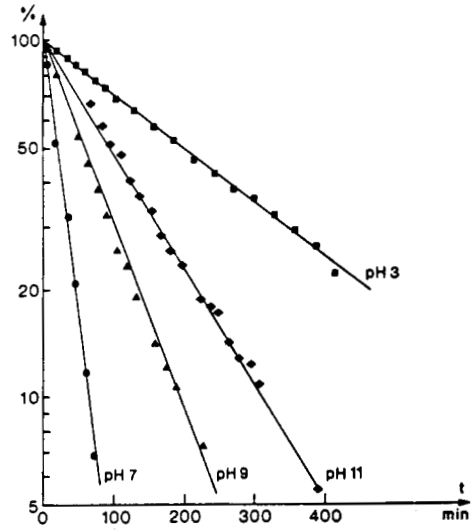


Figure 7. Rates of AcP hydrolysis in the presence of macrocycle **1** at different pH values. Semilogarithmic plots of the percentage of remaining AcP vs. time (min) at pH 3, 7, 9, and 11 in D_2O/H_2O 1/9 solution containing initially 0.03 M AcP and 0.03 M **1** at 40 °C.

consumption was accelerated by addition of 1 equiv of **1** except at pH 3, where it was slightly inhibited (Figure 6) and showed apparent first-order behavior in the pH range investigated (Figure 7).

In the presence of a fivefold excess of AcP, macrocycle **1** acted until all substrate had been consumed. At 84 °C, the plot of AcP concentration versus time gave a straight line with a correlation of 0.999 between four collected points. The slope of this line gave a zero-order observed rate constant $k^0_{obsd} = 13.5\ min^{-1}\cdot L\cdot mol^{-1}$. The calculated first-order rate constant (k') obtained by $k' = k^0_{obsd}/[\mathbf{1}]$ was 0.45 min^{-1}, which is smaller than the observed first-order rate constant at the same pH and temperature in 1/1 **1**/AcP conditions ($k_{obsd} = 0.84\ min^{-1}$). The difference could be due to inhibition of AcP consumption by P and PP produced in the course of the reaction; this effect may also explain the nonlinearity of the plot of [AcP] versus time for data obtained at 40 °C and pH 7.

Inhibition Experiments. The reaction of AcP in the presence of compound **1** and of different competitively bound anions (AMP^{2-}, SO_4^{2-}, $C_2O_4^{2-}$, HPO_4^{2-}) was followed by ^{31}P NMR at pH 7, 40 °C. The results are shown in Table II: in all cases, the observed first-order rate of AcP consumption was significantly decreased; the formation of both **1**-PN and PP as well as the disappearance of **1**-PN were retarded, and the final amounts of PP were markedly increased in presence of P. Figure 4c clearly illustrates these effects in the case of 2 equiv of AMP^{2-} as competing anion. An equimolar mixture of AcP and compound **1** (30 mM) was followed by ^{31}P NMR at pH 7 and 40 °C until no more AcP was present; the solution then contained **1**-PN (19%), P (60%), and PP (21%). To this mixture, 0.15 M (5 equiv) sodium sulfate was added and the pH adjusted to 7. Again the course of the reaction was followed by ^{31}P NMR at 60 °C until no more **1**-PN was present. The composition of the solution was then P (77%) and PP (23%), showing that the additional amount of PP formed was only 2%. When the same experiment was performed without addition of sulfate, the amount of PP was increased by 11% from 21% to 32%.

Saturation Experiments. To six aliquots of a stock solution of **1**-PN (19%) prepared as above were added increasing amounts of sodium phosphate (0.0x mM, x = 1, 2, 3, 4, 5, 7). The pH of each sample was adjusted to 7, and the amounts of PP formed and **1**-PN lost were monitored at 60 and 84 °C by ^{31}P NMR. The relative amount of PP increased with addition of (1–5) × 10^{-2} M sodium phosphate, whereas between 5 × 10^{-2} M and 7 × 10^{-2} M added sodium phosphate, it was constant (Figure 8). The maximum conversion of **1**-PN to PP was ca. 60%.

^{18}O-Labeling Experiments. A mixture of compound **1** (30 mM) and AcP (30 mM) was followed at pH 7, 50 °C by ^{31}P NMR

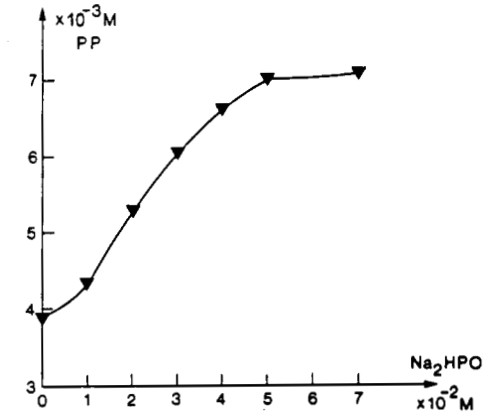


Figure 8. Plot of observed molar concentration of PP produced vs. amounts of P added to **1**-PN solutions generated from 0.03 M AcP and **1** (see text) in D_2O/H_2O 1/9 at 84 °C and pH 7.

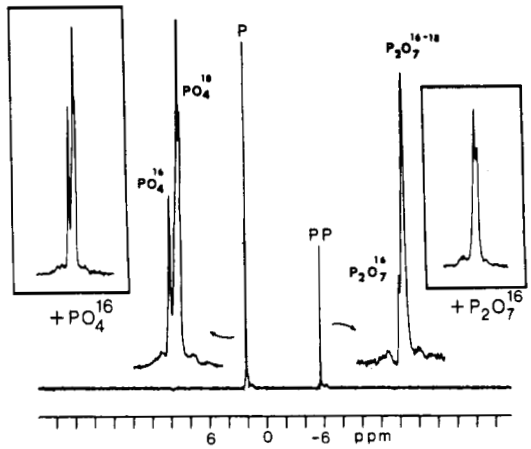


Figure 9. 162-MHz ^{31}P NMR spectra of ^{18}O-labeled PP and P. (^{16}O,^{18}O)-Pyrophosphate was generated by addition of labeled phosphate (~80% total ^{18}O enrichment) to a solution of isolated **1**-PN (see text) at pH 7 and 50 °C in D_2O/H_2O 1/9. The inserts show the expanded signals of unlabeled and labeled P and PP together with the signals obtained by addition of unlabeled P and PP for identification purposes.

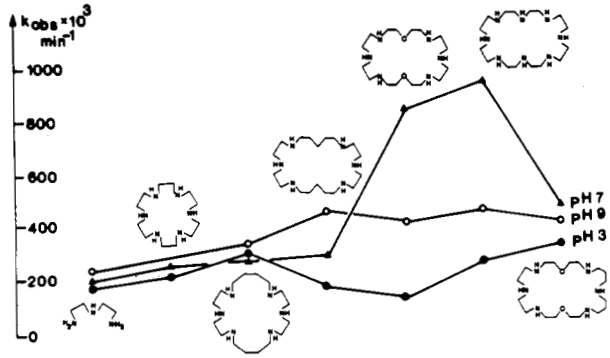


Figure 10. Structural effect on the rate of AcP hydrolysis in the presence of various polyamines in D_2O/H_2O 1/9 solution containing initially 0.03 M AcP and 0.03 M polyamine at 84 °C; pH 3, 7, and 9.

until no more AcP was present; then it was cooled to 25 °C and the pH was raised to 10.5. Addition of 1.62 mmol of $BaCl_2$ precipitated all phosphate-containing compounds except the phosphorylated macrocycle **1**-PN (^{31}P NMR observation). The solution was filtered and 50 mmol of ^{18}O-labeled potassium dihydrogen phosphate (~80% enrichment), prepared as described in ref 20, was added. The solution was again filtered and the mixture was incubated at pH 7 and 50 °C for 15 min. ^{18}O-Labeled pyrophosphate was obtained, which was identified by ^{31}P

(20) Risley, J. M.; Van Etten, R. L. *J. Labelled Comp.* **1978**, *15*, 533–538.

Table II. Inhibition of AcP (30 mM) Reaction and of **1**-PN and PP Formation in the Presence of Compound **1** (30 mM) in H_2O/D_2O 9/1 at pH 7 and 40 °C

	inhibitor[a]						
	none	AMP^{2-}	AMP^{2-}[b]	SO_4^{2-}	$C_2O_4^{2-}$	P^{2-}	P^{2-}[c]
$k_{obsd} \times 10^3$ min^{-1}	36	28.0	19.8	24.3	23.9	24.1	12.8
% **1**-PN (0.5 h)[d]	42	39	32	35	35	31	22
% **1**-PN (1 h)[d]	39	49	44	34	38	38	32
% **1**-PN (4 h)[d]	4	22	33	7	8	14	14
% **1**-PN_{max}[e]	43	50	49	37	38	38	34
% PP (0.5 h)[f]	4	0	0	1	2	1	5
% PP (1 h)[f]	10	0	1	2	5	6	11
% PP (4 h)[f]	31	15	9	26	30	41	52
% PP_{max}[g]	35	20	20	33	30	59	65

[a] Concentration of inhibitor = 30 mM. [b] Concentration of AMP = 60 mM. [c] Concentration of P = 90 mM. [d] Percentage of **1**-PN present after 0.5, 1, and 4 h of reaction time. [e] Maximum percentage observed. [f] Percentage of PP given in P content (see Experimental Section) after 0.5, 1, and 4 h of reaction time. [g] Maximum percentage of PP present given in P content (see Experimental Section) when all **1**-PN is consumed.

Table III. Observed First-Order Rate Constants ($k_{obsd} \times 10^3$ min^{-1})[a] of AcP (30 mM) Consumption in the Presence of Various Polyamines (30 mM) at Different pH Values in H_2O/D_2O 9/1 at 84 °C

	polyamine											
pH	diethylenetriamine	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
3	190	135		240[b]	321	218	235	210[c]		264		290[d]	338[e]
7	206	840	205		460[f]	254	279	292	154	940	600	190	225
9	243	400					332	457		461		153	

[a] k_{obsd} ±20%. [b] k_{obsd} = 0.334 min^{-1} at pH 5. [c] k_{obsd} = 0.191 min^{-1} at pH 6; 0.299 min^{-1} at pH 8; 0.288 min^{-1} at pH 10. [d] k_{obsd} = 0.186 min^{-1} at pH 8. [e] k_{obsd} = 0.172 min^{-1} at pH 8. [f] At 40 °C, $k_{obsd} \times 10^3$ = 17.3 min^{-1}; disappearance of PN, $k_{obsd} \times 10^3$ = 8.2 and 308 min^{-1} at 40 and 84 °C, respectively.

Table IV. Structural and pH Effects on the Amounts of PN[a] and PP[b] Formed during the Reaction of AcP (30 mM) in the Presence of Various Macrocyclic Polyamines (30 mM) in H_2O/D_2O 9/1 at 84 °C[c]

	pH							
	3		7		8		9	
compd	% PN_{max}	% PP_{max}	% PN_{max}	% PP_{max}	% PN_{max}	% PP_{max}	% PN_{max}	% PP_{max}
4	0	0	42[d,e]	6[d]				
6	0	0	0	0			12[e]	0
7	0	0	0	0	9	0	11[e]	0
9	0	24	35[e]	20			17[e]	15
10			32[e]	14				

[a] Maximum percentage of the intermediate (PN_{max}). [b] Maximum percentage of PP (PP_{max}) given in P content (see Experimental Section) when all PN was consumed. [c] Neither PN nor PP was detected in the presence of **2**, **3**, **5**, **8**, **11**, **12**, and diethylenetriamine. [d] PN_{max} = 38.2% and PP_{max} = 4% at 40 °C. [e] Chemical shift (ppm from external P) of ^{31}P signal: 9.80 (**4**); 10.25 (**6**); 10.31, 10.50 (**7**, pH 8, 9); 9.64, 9.61 (**9**, pH 7, 9); 9.80 (**10**).

NMR due to its isotopically shifted signals[21] (Figure 9).

Structural Effects. The effects of various other macrocyclic polyamines and of diethylenetriamine on the rate of AcP consumption are listed in Table III and represented graphically in Figure 10. Diethylenetriamine as well as compounds **2**, **3**, **5**, **8**, **11**, and **12** showed almost no activity. Compounds **4**, **6**, **7**, **9**, and **10** gave rate enhancement. Compound **9** was even a better catalyst (k_{obsd} = 0.94 min^{-1}) than the reference compound **1** (k_{obsd} = 0.84 min^{-1}) at pH 7 and 84 °C. Among the various compounds **2**–**12** tested, only in the presence of compounds **4**, **6**, **7**, **9**, and **10** was a PN intermediate observed; in no case was PN observed at pH 3 (Table IV). Whereas PP was only formed at pH 7 in the presence of **4** and **10**, compound **9** produced significant quantitites of PP from pH 3 to 9 (Table IV).

Other Phosphorylation Experiments. Although the hydrolysis of creatine phosphate and phosphoenol pyruvate was accelerated in the presence of compound **1**, no PN intermediate was observed during the reaction under the same conditions as with AcP (pH 7, 84 °C).

When added to the intermediate **1**-PN prepared as described above, AMP^{2-}, ADP^{3-}, ATP^{4-}, and glucose 6-phosphate were not phosphorylated at pH 7. However, in the presence of pyrophosphate (30 mM), ca. 2% of the starting AcP was transformed into triphosphate, identified by its ^{31}P NMR signals.

(21) Marschner, T. M.; Reynolds, M. A.; Oppenheimer, N. J.; Kenyon, G. L. *J. Chem. Soc., Chem. Commun.* **1983**, 1289–1290. Reynolds, M. A.; Oppenheimer, N. J.; Kenyon, G. L. *J. Am. Chem. Soc.* **1983**, *105*, 6663–6667.

Discussion

Several studies on the mechanism and catalysis of the hydrolysis of AcP and of related compounds have been performed.[22-25] The rate of hydrolysis was found to be highest at low and high pH where acid- and base-catalyzed processes take place, whereas it was slowest and almost constant in a broad range around neutral pH, from pH ~3 to 10.

The present results show that protonated macrocyclic polyamines such as **1**–**12** markedly affect the rate and the products of AcP hydrolysis, yielding *pyrophosphate* in addition to the usual product phosphate. Analysis of the data should allow us to uncover mechanistic and structural factors which control the course of this catalytic PP synthesis.

As discussed earlier in the case of ATP hydrolysis,[16,17] several factors may contribute to the catalysis of AcP consumption in the presence of protonated macrocyclic polyamines. In the present case, the situation is more complicated since two different products P and PP are obtained and the catalytic effects may act simultaneously but differently on the three overall processes: AcP consumption, P formation, and PP synthesis. After initial binding

(22) Koshland, D. E., Jr. *J. Am. Chem. Soc.* **1952**, *74*, 2286–2292. Park, J. H.; Koshland, D. E., Jr. *J. Biol. Chem.* **1958**, *233*, 986.

(23) (a) Di Sabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4393–4400. (b) Di Sabato, G.; Jencks, W. P. *Ibid.* **1961**, *83*, 4400–4405. (c) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, *108*, 7938–7946.

(24) Philips, D. R.; Fife, T. H. *J. Am. Chem. Soc.* **1968**, *90*, 6803–6809; *J. Org. Chem.* **1969**, *34*, 2710–2714.

(25) Kutz, J. L.; Gutsche, C. D. *J. Am. Chem. Soc.* **1960**, *82*, 2175–2181. Lau, H. P.; Gutsche, C. D. *Ibid.* **1978**, *100*, 1857–1865.

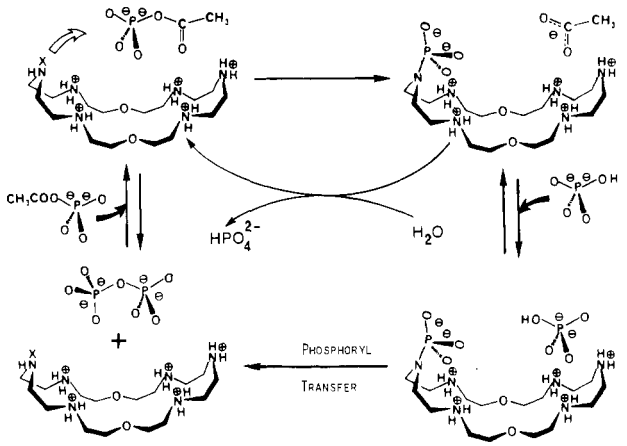


Figure 11. Schematic representation of the catalytic cycle for AcP hydrolysis and PP synthesis by the macrocyclic catalyst **1**. The geometries of the [**1**,AcP] and [**1**-PN,P] complexes and the binding schemes are hypothetical but compatible with the structure, the dimensions, and the binding-site arrangement of all partners.

of AcP to the protonated macrocyclic polyamine, the following three factors may operate within the supramolecular species formed (see also[16,17]): *electrostatic catalysis*, in which binding overcomes electrostatic repulsion and facilitates reaction between electron-rich species, yielding products of higher total charge than the starting AcP; *acid catalysis* at low pH by proton transfer within the bound species; *nucleophilic catalysis* by an unprotonated nitrogen site which undergoes phosphorylation. It is clear that in addition to bound AcP, free AcP will also be present, in amounts depending on pH, and undergo reaction.

Electrostatic and acid catalysis are expected to contribute at low pH and to decrease as the pH rises. The nucleophilic pathway, which can also take advantage of electrostatic interactions, should lead to a maximum in the dependence of AcP consumption rate on pH since it requires at least one unprotonated nitrogen site. At low pH, all sites are protonated; at too high pH, lesser protonation of the macrocycles decreases AcP binding. Furthermore, the detection of a phosphorylated intermediate is of course diagnostic of nucleophile participation.

Most of the present results concern macrocycle **1**; they will serve as a basis for discussing the mechanism of AcP hydrolysis and of PP synthesis. The data obtained for the other macrocycles will be used to specify the effects of structure of the molecular catalyst on the reaction parameters and products.

Macrocyclic polyamine [24]-N_6O_2 (1) was first obtained as a tetra-*N*-tosyl derivative in the course of the synthesis of the bis-tren macrobicycle[26] and later by a more direct route.[27] It is a coreceptor molecule[3] whose properties result from three main features: the macrocyclic structure, the ditopic nature, and two diethylenetriamine subunits.

Ligand **1** has been widely exploited for its ability to form dinuclear metal complexes of bioinorganic interest.[28] On the other hand, when protonated it binds molecular anions[29] and presents properties of bioorganic nature such as binding and hydrolysis of ATP.[16,17]

(26) Lehn, J. M.; Pine, S. H.; Watanabe, E. I.; Willard, A. K. *J. Am. Chem. Soc.* **1977**, *99*, 6766–6768.
(27) Comarmond, J.; Plumeré, P.; Lehn, J. M.; Agnus, Y.; Louis, R.; Weiss, R.; Kahn, O.; Morgenstern-Badarau, I. *J. Am. Chem. Soc.* **1982**, *104*, 6330–6340.
(28) Lehn, J. M. *Pure Appl. Chem.* **1980**, *52*, 2441–2459. Lehn, J. M. In *Frontiers in Chemistry*; Laidler, K. J., Ed.; Pergamon: Oxford, 1982; pp 265–272. Coughlin, P. K.; Dewan, J. P.; Lippard, S. J.; Watanabe, E. I.; Lehn, J. M. *J. Am. Chem. Soc.* **1979**, *101*, 265–266. Motekaitis, R. J.; Martell, A. E.; Lecomte, J. P.; Lehn, J. M. *Inorg. Chem.* **1983**, *22*, 609–614. Lecomte, J. P.; Lehn, J. M.; Parker, D.; Guilhem, J.; Pascard, C. *J. Chem. Soc., Chem. Commun.* **1983**, 296–298. Coughlin, P. K.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 2328–2336. Tai, A. F.; Margerum, L. D.; Valentine, J. S. *Ibid.* **1986**, *108*, 5006–5008.
(29) Hosseini, M. W. Thèse de Doctorat-ès-Sciences, Université Louis Pasteur, Strasbourg, 1983.

Catalysis of AcP consumption by macrocycle **1** mediates both hydrolysis to P and PP formation; it involves an intermediate, the phosphorylated macrocycle **1**-PN. The results described above give insight into the mechanistic steps of these two catalytic processes.

Catalytic Cycle for Pyrophosphate Synthesis by Macrocycle 1. The following sequence of steps may be proposed for the mechanism of PP generation from AcP (Figure 11): (a) substrate (AcP) binding by the protonated molecular catalyst **1** (protons omitted)

$$\text{AcP} + \mathbf{1} \rightleftharpoons [\mathbf{1},\text{AcP}]$$

(b) phosphoryl transfer within the supramolecular complex, giving the phosphorylated intermediate **1**-PN

$$[\mathbf{1},\text{AcP}) \rightarrow \mathbf{1}\text{-PN} + \text{AcO}^-$$

(c) binding of HPO_4^{2-} (P) by the **1**-PN intermediate

$$\mathbf{1}\text{-PN} + \text{P} \rightleftharpoons [\mathbf{1}\text{-PN},\text{P}]$$

(d) phosphoryl transfer to P within the second supramolecular complex, giving PP

$$[\mathbf{1}\text{-PN},\text{P}] \rightarrow [\mathbf{1},\text{PP}]$$

(e) product dissociation and release of the free receptor for a new catalytic cycle

$$[\mathbf{1},\text{PP}) \rightleftharpoons \mathbf{1} + \text{PP}$$

Step a: Substrate Binding. The protonated macrocycle **1** forms complexes with a variety of anions by interaction between the negatively charged centers of these substrates and the positively charged ammonium sites located in the receptor framework. The stability of these complexes is generally higher the larger the number of interacting charges; thus, AMP^{2-}, ADP^{3-}, and ATP^{4-} give stability constants in the range log $K_s \sim 10^3$–10^{11}. Because of its reactivity, the stability of the AcP complex could not be determined. However, since both species have the same charge, AcP ($pK_{a1} = 4.95$) should form with protonated **1** complexes of stability similar to those of AMP,[16,29] with binding constants of about 10^2–10^4 L·mol^{-1} for the [**1**-4H$^+$,AcP^{2-}] and [**1**-5H$^+$,AcP^{2-}] complexes, which should predominate at pH 7. Complexation was demonstrated by the observation of different chemical shifts for the ^{31}P NMR signal in the absence (−1.44 ppm) and in the presence (−2.00 ppm) of compound **1** (H_2O/D_2O 9/1, pH 7, 40 °C). With an excess of AcP over compound **1**, only a single line was observed by ^{31}P NMR, indicating that complex formation and dissociation were fast on the NMR time scale.

The rate data (first-order kinetics; Figure 7) agree with the AcP reaction taking place predominantly via preformed [**1**-*n*H$^+$,AcP] complexes as the reactive species. This is also supported by the observation of rate saturation when compound **1** is present in excess (2 equiv). Furthermore, the rate of AcP consumption in the presence of **1** must be decreased by addition of competitively bound anions which inhibit AcP binding, as was indeed observed on addition of AMP, sulfate, oxalate, and phosphate (Table II).

Step b: AcP Consumption and Phosphoryl Transfer. Formation and Reactivity of the 1-PN Intermediate. In addition to electrostatic effects due to strong charge–charge interactions (as in the case of ATP hydrolysis[16,17]), binding of AcP to **1**-*n*H$^+$ may also provide conditions for nucleophilic catalysis of AcP consumption. Indeed, whereas the rate of the uncatalyzed AcP hydrolysis is almost constant and minimum between pH ca. 5 and 10,[22] the observed first-order rate constant of AcP consumption in the presence of **1** is maximum at pH 7, with an acceleration factor at 40 °C of ca. 5 (Figure 6).

The observation of a phosphoramidate intermediate is a direct indication for the occurrence of nucleophilic catalysis, leading to phosphorylation of **1** to give **1**-PN, whose structure was assigned by NMR experiments (see above). The central location of the phosphoramidate within a diethylenetriamine binding subunit of **1** agrees with the fact that this site has by far the lowest pK_a value.[30] Furthermore, several diamine monocations are found

to be more reactive toward monosubstituted phosphate derivatives than the more basic free diamines.[31] The identity of the ^{31}P NMR chemical shifts may be taken as an indication that the present **1**-PN intermediate is the same as that detected in the hydrolysis of ATP catalyzed by macrocycle **1**, thus confirming the previous observations and mechanistic discussion.[16,17] No acylation of **1** was detected by ^1H NMR at pH 7 and 11, whereas in the presence of primary and secondary amines, AcP gives the corresponding acetamides.[23] The rate of **1**-PN disappearance (Table I) was first order (Figure 5) and decreased from pH 6 to 9, a behavior which resembles that of simple phosphoramidates.[32]

The addition of anionic species retarded the formation of **1**-PN but did not much affect the maximum amount of observed **1**-PN_{max}, which is reached much later (about 100 min of reaction time in the presence of 2 equiv of AMP^{2-} (Figure 4c) as compared to ca. 40 min (Figure 4a)). This agrees with the fact that these species compete with both AcP and P binding, thus decreasing the rate of formation as well as of disappearance of **1**-PN (Table II; Figure 4c).

In view of the efficiency of **1**-PN formation and the characteristic pH dependence of AcP consumption (Figure 6), nucleophilic catalysis is probably predominant. At pH 3, the AcP consumption was slowed down in the presence of **1**. A possible explanation could be that complexation should decrease the pK_{a1} of AcP (AcP^-/AcP^{2-}), so that the amount of AcP^{2-} (less reactive than AcP^-) present at pH 3 is increased in the presence of compound **1**; furthermore, since complexes [**1**-$6H^+$,AcP^-] and [**1**-$6H^+$,AcP^{2-}] should predominate at this pH, the lower rate in the presence of **1** might reflect some inhibition by hydrogen bonding to the protonated catalyst, compared to nucleophilic catalysis at higher pH. The observation of ca. 6% PP nevertheless indicates that some **1**-PN must have been formed, although it was not detected because its rate of consumption increases markedly when the pH decreases from 7 to 3. In agreement with a larger contribution from nucleophilic catalysis, increasing the pH from 3 to 7 produces higher percentages of **1**-PN and larger acceleration factors (Table I), along with the increase in proportion of complexes of the type [**1**-$5H^+$,AcP^{2-}] and [**1**-$4H^+$,AcP^{2-}] which possess at least one unprotonated nitrogen. Between pH 7 and 9, the amount of [**1**-$3H^+$,AcP^{2-}] complex is expected to increase; since a high percentage of **1**-PN and significant acceleration factors were still obtained (Figure 6; Table I), the stability constant of this complex should be high enough to allow formation of the supramolecular complex leading to phosphorylation of the macrocycle; on the other hand, a much slower hydrolysis of **1**-PN leads to its accumulation. At pH 11, the unprotonated compound **1** is dominant so that complexation does not occur and neither acceleration nor **1**-PN was observed; this also indicates that AcP does not phosphorylate the unprotonated macrocycle, further stressing the requirement for protonated sites in the **1**-PN formation step. However, partial protonation of the polyamine is not sufficient since diethylenetriamine is not phosphorylated at pH 7 (where it is diprotonated), pointing to the crucial role of the structure of the polyamine catalyst (see below).

Step c: Phosphate Binding and Pyrophosphate Formation. The formation of PP is by far the most significant feature of the reactions studied here, since it demonstrates catalysis of a bond-making process. PP may in principle be generated via several pathways (protonations omitted): reaction of AcP with **1**-PN yielding a pyrophosphoramidate **1**-PPN, which is rapidly hydrolyzed to PP

$$\text{1-PN} + \text{AcP} \rightarrow \text{1-PPN} \xrightarrow{H_2O} [\text{1,PP}] \quad (1)$$

intermolecular reaction of two intermediates **1**-PN giving **1**-PPN, followed by hydrolysis

$$\text{1-PN} + \text{1-PN} \rightarrow \text{1} + \text{1-PPN} \xrightarrow{H_2O} [\text{1,PP}] \quad (2)$$

double phosphorylation of **1**, followed by internal phosphoryl transfer giving **1**-PPN and hydrolysis

$$\text{1-PN} + \text{AcP} \rightarrow \text{1-(PN)}_2 \rightarrow \text{1-PPN} \xrightarrow{H_2O} [\text{1,PP}] \quad (3)$$

phosphorylation of unbound P by **1**-PN

$$\text{1-PN} + \text{P} \rightarrow \text{1} + \text{PP} \quad (4)$$

complexation of P by protonated **1**-PN, followed by phosphoryl transfer within the supramolecular species

$$\text{P} + \text{1-PN} \rightarrow [\text{1-PN,P}] \rightarrow [\text{1,PP}] \quad (5)$$

The formation of labeled PP from isolated **1**-PN and ^{18}O-enriched P (Figure 9) rules out processes 1, 2, and 3 as major pathways. In addition, no diphosphorylated macrocycle **1** was detected and the formation of PP was still observed, concomitantly with **1**-PN disappearance, after all AcP had reacted (Figures 1 and 4a). Strictly speaking, however, minor contributions from pathways 1, 2, and 3 to the overall AcP → PP transformation cannot be totally excluded, if these processes are fast.

Formation of PP via route 4 would be bimolecular, as in the synthesis of dibenzyl pyrophosphate from benzyl phosphoramidate in organic medium.[33] The addition of 1 or 2 equiv of AMP markedly decreased the PP formation rate and the total amounts of PP obtained, although **1**-PN_{max} was the same as in the absence of AMP (Table II; Figure 4c); this is a strong indication that AMP hinders phosphorylation of P, probably by competitive binding. Addition of 1 equiv of sulfate or oxalate also retarded PP generation but did not much decrease PP_{max} (Table II). Thus AMP is a stronger inhibitor than sulfate and oxalate and, furthermore, diverts the fate of **1**-PN toward hydrolysis to P. The formation of PP could even be completely stopped by addition of 5 equiv of sulfate after all AcP had reacted. On the other hand, on addition of increasing amounts of sodium phosphate to a solution of the **1**-PN, the increase in amounts of PP formed showed saturation behavior reaching a maximum of PP generation of ca. 60% in the conditions of Figure 8. It is clear that the doubly charged HPO_4^{2-} substrate should be bound much more strongly by protonated **1**-PN than the acetate anion coming from AcP.

These results speak in favor of pathway 5, but they do not allow us to exclude entirely contribution from the intermolecular pathway 4. According to route 5, the two diethylenetriamine subunits of the ditopic coreceptor **1** cooperate, one of them forming the PN intermediate, and the other the protonated subunit binding the phosphate substrate; thus, the generation of PP from AcP in the presence of **1** would proceed via two successive intramolecular phosphoryl transfer reactions occurring within two different supramolecular complexes [**1**,AcP] and [**1**-PN,P].

The fact that at pH 9 AcP produces in the presence of **1** a large amount of **1**-PN but no PP may result from two effects: (i) at that pH the number and location of protonated ammonium sites (probably 3)[30] could be such that the first phosphorylation yielding **1**-PN takes place, but binding of P to incompletely protonated **1**-PN being too weak, the second phosphoryl transfer to P becomes very inefficient; (ii) PN hydrolysis is expected to be much slower at basic pH and thus **1**-PN accumulates. Because of the reactivity of **1**-PN it was not possible to determine its pK_a's and its ability to bind P. Less than 2% decomposition of **1**-PN occurred in 5 h at 40 °C at pH 9. Phosphoramidate ($H_2NPO_3^{2-}$) itself does not hydrolyze at 60 °C above pH 10.[32a]

During AcP consumption in the presence of **1**, the formation of PP is in competition with the hydrolysis of **1**-PN to P and **1**, and PP generation can only occur after some P has been produced, so that ca. 30% conversion of AcP to PP is already quite remarkable. Although no PP was detected in the absence of **1**, solvolysis of concentrated aqueous AcP solutions (0.4 M) has been reported to give traces of PP.[23] On the other hand, AcP yields

(30) Dietrich, B.; Hosseini, M. W.; Lehn, J. M.; Sessions, R. B. *Helv. Chim. Acta* **1983**, *66*, 1262–1278.
(31) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209–3216.
(32) (a) Chanley, J. D.; Feageson, E. *J. Am. Chem. Soc.* **1963**, *85*, 1181–1190. (b) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1965**, *87*, 3199–3209. (c) Benkovic, S. J.; Sampson, E. J. *Ibid.* **1971**, *93*, 4009–4016.
(33) Clark, V. M.; Warren, S. G. *Proc. Chem. Soc., London* **1963**, 178–179.

PP in concentrated aqueous salt solutions when the activity of water is considerably reduced. The molar percentage of PP synthesized by macrocycle **1** from 0.03 M AcP in the presence of 0.09 M Na_2HPO_4 (pH 7, 40 °C) was 32.5% (obtained from PP_{max} 65% (Table II); see Experimental Section); this may be compared to the formation of about 9% PP from 0.046 M AcP in the presence of 0.25 M Na_2HPO_4 at 7.0 M sodium ion (pH ~ 7, 54.4 °C).[23c] Phosphoramidate esters are used in organic medium as reagents for the synthesis of pyrophosphate derivatives.[34] Formation of PP occurs by phosphoryl transfer from ATP catalyzed by divalent metal ions.[35]

In addition to the PP generation pathways 1–5, another route 6 not involving the **1**-PN intermediate could contribute as long as there is AcP present. It would involve simultaneous binding of AcP and P to protonated **1** followed by phosphoryl transfer within the three-component supramolecular species thus formed:

$$\mathbf{1} + \text{AcP} + \text{P} \rightleftharpoons [\mathbf{1},\text{AcP},\text{P}] \rightarrow [\mathbf{1},\text{AcO}^-,\text{PP}] \qquad (6)$$

In such a process, **1** would provide electrostatic interaction and collect the two reactants. These factors are molecular analogues of the electrostatic and anion bridging role assigned to sodium ions in the course of PP formation from AcP and P in concentrated aqueous salt solutions.[23c]

In conclusion, in the PP generation process, macrocycle **1** acts both as electrostatic catalyst, shielding the charge repulsion between the two reactants, and as cocatalyst, bringing the two reactants together; both effects facilitate phosphoryl transfer to P, possibly together with a more subtle orientational factor linked to the structure of the macrocycle **1** and of the subsequent supramolecular species formed during the reaction (see below). Thus, compound **1** provides in aqueous solution suitable conditions at the molecular level for phosphoryl transfer from AcP to **1** yielding **1**-PN, which in turn accomplishes a phosphoryl transfer to P. Either or both phosphoryl transfer processes may involve the formation of a more or less "free" metaphosphate intermediate.[17b]

Binding and Hydrolysis of PP by Macrocycle 1. The amount of PP observed will also depend on its own reaction with macrocycle **1**. Titration of PP by **1** followed by ^{31}P NMR spectroscopy gave a stoichiometry of 1:1 for the complexes present at pH 7.5 (in H_2O/D_2O 9/1 at 25 °C).[29] The hydrolysis of PP (uncatalyzed, $k_{obsd} = 6 \times 10^{-4}$ and 2×10^{-4} min^{-1} at pH 3.5 and 7.5, respectively, in H_2O, 70 °C[36]) was catalyzed by macrocycle **1** ($k_{obsd} = 97 \times 10^{-4}$ and 22×10^{-4} min^{-1} at pH 3 and 7, respectively, in H_2O/D_2O 9/1 at 80 °C[16]). Therefore, since the PP formed in the course of the AcP reaction slowly gives P, the amount of PP actually generated must be higher than the observed one.

Catalytic Cycle for AcP Hydrolysis. As already mentioned, macrocycle **1** also catalyzes AcP hydrolysis to P, and this reaction is in competition with PP formation. Its mechanism may involve pathways and effects similar to those considered for ATP hydrolysis in the presence of **1**:[16,17] (a) overall electrostatic catalysis, since the products HPO_4^{2-} and AcO^- have larger total charge than the reactant AcP^{2-}; (b) catalysis of direct addition of water to AcP bound to **1**, which could in principle occur at the phosphoryl and/or at the acetyl centers;[22] (c) nucleophilic catalysis of AcP hydrolysis via formation of the common intermediate **1**-PN which yields either P or PP by phosphoryl transfer to water or to P, respectively.

The catalytic cycle corresponding to path c is also indicated in Figure 11. The pH dependence of **1**-PN hydrolysis may be considered to be similar to that of simple phosphoramidates, the rate decreasing with increasing pH.[32] However, the presence of protonizable nitrogen sites next to the $N-PO_3^{2-}$ group and of the second diethylenetriamine unit could activate phosphoryl transfer to water at low pH and perhaps hinder it at high pH, with respect to a simple phosphoramidate $R_2N-PO_3^{2-}$.

Structural Effects on PN and PP Generation. Taking macrocycle **1**, the most extensively studied catalyst, as reference compound, a series of related macrocycles was employed in order to investigate the effect of structural modifications which may affect the basicity and nucleophilicity of the nitrogen sites as well as the geometry of the supramolecular species involved (Table IV). The formation of a phosphoramidate species was detected by the observation of a high-field ^{31}P NMR signal (~+10 ppm); however, the exact structure of these PN species was not determined. pH effects on AcP consumption rates may give indication on the catalytic factors provided by a given macrocycle (see above).

Modification of the Binding Subunits. Methylation of all six secondary amino groups gave compound **2**, which had almost no effect at pH 7 on the rate of AcP consumption, and neither phosphoramidate nor PP was observed. Thus, replacing secondary by tertiary amines inhibits the nucleophilic catalysis.

Attachment of a $CH_2CH_2NH_2$ side chain to the six amino groups of **1** yielded compound **3**, which contains primary as well as tertiary amines and possesses rather complicated protonation features. This compound showed some effect on the AcP reaction (possibly of electrostatic nature) but neither phosphoramidate nor PP was detected.

Modification of one of the binding subunits was achieved in compound **4**. Due to the respective protonation features of the diethylenetriamine and dipropylenetriamine subunits,[30] [**4**-$5H^+$,AcP^{2-}] complexes should be predominant at pH 7 (^{31}P = −2.70 ppm), the unprotonated amine being the central site of the diethylenetriamine group. This compound, in addition to electrostatic catalysis, showed nucleophilic catalysis. Indeed, the rate of AcP consumption in the presence of **4** was higher at pH 7 than at pH 3 (Table III) and 42% of phosphoramidate was obtained (Table IV). Despite this rather large amount of intermediate, the amount of PP observed was quite low (6%). This could be due either to low production or relatively fast hydrolysis of PP. The rate of PP hydrolysis catalyzed by compound **4**, determined independently at pH 7 (k_{obsd} = 0.0062 min^{-1} at 80 °C), was faster than in the presence of compound **1** (k_{obsd} = 0.0022 min^{-1}, pH 7, 80 °C). However, the ratio (percentage of PP observed in the presence of **1** over percentage of PP observed in the presence of **4**) = 35%/6% = 5.8 is much larger than the ratio of the rate constants for PP hydrolysis (2.8). Since both **1** and **4** yield similar amounts of PN, the low percentage of PP formed in the presence of **4** may be taken as an indication for a structural effect on the second phosphoryl transfer step to phosphate, which may act on binding and/or location of the acceptor P.

Modification of the Intersubunit Bridges. Modifications of the chains bridging the two diethylenetriamine subunits in **1** were achieved in macrocycles **5**–**8**. In compounds **5** and **6**, an 18- and a 22-membered macrocycle, respectively, the distance separating the two subunits is shorter than in the reference compound **1**, while it is comparable in **7** and longer in **8**. The rates of AcP consumption were found to be slower in the presence of compounds **5**–**8** than in the presence of **1** (Table III), the large macrocycle **8** being the least active. Although none of these compounds led to PP generation at any pH studied, significant amounts of a PN intermediate were formed only with **6** and **7** (whose structure is closest to **1**), not with **5** and **8**, and at a higher pH than with **1** (Table IV). This agrees at least qualitatively with the requirement for a free nitrogen site, since **6** and **7** are expected to be more basic than **1**. Furthermore, the rate of AcP consumption in the presence of **6** and **7** was highest at basic pH, in accordance with nucleophilic catalysis via the PN derivative.

Modifications of both Subunits and Bridges. Compound **9** can be considered as a close analogue of **1** where the two oxygen atoms are replaced by two nitrogens, increasing the number of potential binding and nucleophilic sites. This compound shows similar activities (nucleophilic and electrostatic catalysis) as compound **1** (Table III). In the presence of **9**, maximum amounts of 35% at pH 7 and 17% at pH 9 of a PN intermediate were generated.

(34) Clark, V. M.; Kirby, G. W.; Todd, A. *J. Chem. Soc.* **1957**, 1497–1501. Chambers, R. W.; Khorana, H. G. *J. Am. Chem. Soc.* **1958**, *80*, 3749–3752. Chambers, R. W.; Moffatt, J. G. *Ibid.* **1958**, *80*, 3752–3756. Moffatt, J. G.; Khorana, H. G. *Ibid.* **1958**, *80*, 3756–3761.

(35) Lowenstein, J. M. *Biochem. J.* **1958**, *70*, 222–230. Nelson, N.; Racker, E. *Biochemistry* **1973**, *12*, 563–566.

(36) Turyn, D.; Baumgartner, E.; Fernandez-Prini, R. *Biophys. Chem.* **1974**, *2*, 269–272.

Of special interest was the observation that, whereas no PP was formed at pH 9 in the presence of **1**, compound **9** still gave 15% of PP. On the other hand, although no intermediate was observed at pH 3, the percentage of PP produced (24%) was highest (Table IV), indicating an efficient transfer from PN to P and/or the operation of a reaction not involving a PN species, such as pathway 6 above. The rates of PP hydrolysis in the presence of **9** were found to be k_{obsd} = 0.00299 and 0.0096 min^{-1} at pH 3 and 7 (80 °C), respectively. Thus, compound **9**, being about 4 times more efficient in the hydrolysis of PP than compound **1**, must also be a better catalyst for its formation, since comparable amounts of PP were produced by **1** and **9** at pH 7.

Comparing macrocycles **6**, **7**, and **9**, we see that they yield about the same amount of PN at pH 9, but PP is produced only with **9**. This may result from the presence of more nitrogen sites in **9** giving a higher degree of protonation at pH 9[37] and/or allowing better binding and positioning of P for phosphoryl transfer. Similarly, **1**, which has lower pK_a's, yields even more PN than **9** but does not give PP at pH 9.

In compound **10**, the number of potential binding and nucleophilic sites is increased from 6 (compound **1**) to 10. This compound produces at pH 7 up to 32% of PN intermediate and 14% of PP. Since the protonation features of the complexes formed with AcP are very complicated due to the large number of amino groups, structural effects are difficult to discuss. It may be noted that compound **10** probably possesses several unprotonated nitrogen sites and might undergo more than one phosphorylation; however, only a single ^{31}P NMR signal was observed. Furthermore, the formation of PP also indicates that the structural requirements are not so strict; possibly, the presence of several protonated nitrogen sites is sufficient for inducing PP formation by activating the PN group for transfer and bringing the P acceptor into its proximity.

In compounds **11** and **12**, the diethylenetriamine subunits are replaced by dipropylenetriamine moieties. Because of the higher pK_a values of these groups, macrocycles **11** and **12** are fully protonated at pH 7.[30] Consequently, no nucleophilic catalysis can take place and no PN intermediate nor PP was observed. In agreement with electrostatic and acid catalysis, the rate of AcP consumption in the presence of **11** and **12** was highest at low pH (Table III).

In summary, the formation of both the intermediate phosphorylated macrocycles PN and the product PP is under marked structural control, PP being appreciably more dependent on structural variations than PN. Comparison of the results obtained for the various macrocycles **1–12** leads to considerations in agreement with the general features of the mechanism of AcP consumption and of PN and PP generation: (i) both amine basicity and macrocycle geometry play a role; (ii) PN formation is more sensitive to pK_a and pH since it requires unprotonated nitrogen site(s); (iii) PP synthesis depends on both basicity, which determines protonation and P binding, and geometry, which controls positioning of the substrate P. In addition, since the generation of pyrophosphate is in competition with its hydrolysis, both catalyzed by the same receptor catalyst, accumulation of the PP product implies that its rate of formation must be higher than its rate of hydrolysis.

Conclusion

Extending our earlier work on supramolecular catalysis,[2,4] the present study shows that macrocyclic polyamines are able to catalyze not only bond-breaking but also bond-making reactions. The synthesis of pyrophosphate from acetyl phosphate mediated by macrocyclic polyamines in water represents such a process of covalent bond formation taking place via supramolecular species. The catalytic cycle may involve binding of an active substrate (AcP = source) by the receptor catalyst; formation of a covalent intermediate, the phosphorylated catalyst (phosphorylating agent); binding of a second substrate (P = phosphoryl acceptor); and synthesis of the product (PP) by a second intramolecular phosphoryl transfer. This requires macrocyclic polyamines that possess simultaneously binding (ammonium) and transformation (amine) sites properly located in the molecular framework. Such is the case for coreceptor molecules, which may function as cocatalysts if their subunits cooperate not only for binding of substrate(s) but also for transformation of the bound species.

The successive generation of a PN intermediate and of PP by such simple receptor molecules as **1** and **9**, for instance, is quite remarkable in view of the rather tight set of binding and reactivity features required: proper acid–base and structural parameters allowing the coexistence of nucleophilic and protonated species for AcP binding and phosphoryl transfer; single phosphorylation and central location of the phosphoryl group; the presence in the PN intermediate of a phosphate binding site, in appropriate location with respect to the phosphorylated center; and phosphoryl transfer to phosphate in competition with transfer to water.

The steps involved in the synthesis of pyrophosphate have analogies with those taking place in enzyme-catalyzed phosphoryl transfers occurring via a phosphorylated enzyme intermediate.[13] A pyrophosphate-dependent acetate kinase catalyzes the same overall process AcP + P ⇌ PP + acetate.[38] One may also note that PP replaces ATP in a number of enzyme-catalyzed reactions involved in biological energy conservation and transfer in lower organisms;[38] furthermore, it may play a role in the molecular evolution of biological energy conversion,[39] starting with its formation in nonenzymatic prebiotic conditions,[40] which might have led to its entry into early metabolism. Compound **1** and compounds **4**, **9**, and **10** display both phosphatase and kinase type activity, depending on whether the phosphorylated intermediate transfers its phosphoryl group either to water or to phosphate. The latter bond-making, *protokinase-like* process is of special significance, since it extends supramolecular reactivity to *cocatalysis*, by which the supramolecular entities formed by coreceptor molecules mediate specific *synthetic* reactions.

Experimental Section

General. Melting points (mp) are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker SY200 spectrometer. Chemical shifts are given in ppm with respect tetramethylsilane in $CDCl_3$ and *tert*-butyl alcohol in D_2O as internal standards. Mass spectra (MS) and elemental analysis were performed by the Service de Spectrométrie de Masse and by the Service de Microanalyse, Institut de Chimie, Strasbourg.

Materials. Compounds **1**,[28] **8**,[9] and **12**[30] were prepared previously. The commercially available chemicals used were of reagent grade. K^+,Li^+ salt of acetyl phosphate was purchased from Boehringer, Mannheim. Compound **5** was commercially available from Aldrich Chemical Co. as its trisulfate salt and was converted into its hexahydrochloride by passage over an anion-exchange resin.

Synthesis of Macrocyclic Polyamines. General Comments. Compound 2 was obtained as a viscous colorless liquid by permethylation of **1** by the Eschweiler–Clark reaction (71% yield): ^{13}C NMR $CDCl_3$ 65.3 (CH_2O); 57.4, 53.6, 52.7 (CH_2N); 41.7, 41.5 (CH_3). Anal. Calcd for $C_{22}H_{56}O_2N_6Cl_6H_2O$: C, 39.58; H, 8.75; N, 12.59. Found: C, 39.52; H, 8.73; N, 12.56.

Compound 3 was obtained by treatment of **1** with tosylaziridine,[41–43] yielding the protected compound **3a**, which was deprotected by treatment with 33% HBr/AcOH/phenol.[30,44]

For macrocyclic **compounds 4**, **6**, **7**, **9**, and **10**, the key step, the cyclization, was achieved by condensation of an α,ω-bis(*p*-toluenesulfonamide) with an α,ω-dimesylate in dimethylformamide (DMF) following the methods described for the synthesis of diazacyclodecadiene[45] and of

(37) Bianchi, A.; Mangani, S.; Micheloni, M.; Nanini, V.; Orioli, P.; Paoletti, P.; Seghi, B. *Inorg. Chem.* **1985**, *24*, 1182–1187.

(38) Reeves, R. E.; Guthrie, J. D. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1389–1395. Reeves, R. E. *Trends Biochem. Sci* **1976** (Mar), 53–55.

(39) Baltscheffsky, H.; Lundin, M.; Luxemburg, C.; Nyrēn, P.; Baltscheffsky, M. *Chem. Scr.* **1986**, *26B*, 259–262.

(40) Seel, F.; Klos, K.-P.; Recktenwald, D.; Schuh, J. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1986**, *41B*, 815–824.

(41) Gauss, W.; Moser, P.; Schwarzenbach, G. *Helv. Chim. Acta* **1952**, *35*, 2359–2363.

(42) Dietrich, B.; Hosseini, M. W.; Lehn, J. M.; Sessions, R. B. *Helv. Chim. Acta* **1985**, *68*, 289–299.

(43) Martin, A. E.; Bulkowski, J. E. *J. Org. Chem.* **1982**, *47*, 415–418.

(44) Snyder, H. R.; Heckert, R. E. *J. Am. Chem. Soc.* **1952**, *74*, 2006–2009.

(45) Feigenbaum, A.; Lehn, J. M. *Bull. Soc. Chim. Fr.* **1973**, 198–202.

poly(heteromacrocycles)[46] but in the presence of cesium carbonate (Cs_2CO_3).[47]

Compound 4 was prepared following a synthetic strategy similar to that used for the synthesis of **1**.[27] The reaction of **13a**[27] with 2-(2-chloroethoxy)ethanol gave **13b**,[27] which was transformed into its dimesylate derivative **13c** by treatment with methanesulfonyl chloride. Cyclization of compounds **13c** and **13d**, prepared as previously described,[42] was performed in DMF at 95 °C in the presence of Cs_2CO_3, yielding macrocyclic compound **4a**. Deprotection in 33% HBr/AcOH/phenol[30,44] gave the compound **4**-6HBr, which was converted to the free polyamine **4** by passage over a Dowex column in its basic form. Compound **4** was stored as **4**-6HCl.

Compounds 6 and 7 were prepared by modification of the published procedures.[43] The use of Cs_2CO_3[9,30,47] in the cyclization step increased significantly the yield of compounds **6a** and **7a**, 70% yield versus 25% yield[43] for compound **6a** and 60% yield versus 20% yield for compound **7a**. Detosylation with 33% HBr/AcOH/phenol yielded **6**-6HBr and **7**-6HBr, respectively, in yields higher than those reported for treatment with concentrated H_2SO_4.[43]

Compound 9 was prepared by a slight modification of the published procedures.[37,46]

Compound 10 was prepared following a strategy similar to that used for the synthesis of **9**.[37,46] Tosylation of tetraethylenepentaamine gave compound **14a**, which was the common starting material for the two chains used in the cyclization step. Treatment of **14a** with 2-bromoethanol in DMF in the presence of K_2CO_3 gave diol **14b**, which was converted into bis(mesyloxy) derivative **14c**. Condensation of **14c** with the second cyclization partner **14a** in DMF at 95 °C in the presence of Cs_2CO_3 led to decatosyl macrocycle **10a**. Removal of the tosyl groups was achieved as described above.

1,4,7,13,16,19-Hexaaza-10,22-dioxa-1,4,7,13,16,19-hexakis(2-(*N*-(*p*-tolylsulfonyl)amino)ethyl)cyclotetracosane (3a). In a 250-mL flask compound **1** (0.54 g, 1.5 mmol), tosylaziridine (6.14 g, 31.2 mmol) (prepared as described in ref 42 and 43), and dry $CHCl_3$ (100 mL) were stirred and heated to 50 °C for 12 h. After evaporation to dryness, pure **3a** was obtained as a colorless viscous liquid by chromatography (alumina/MeOH–CH_2Cl_2, 0–1%) (1.75 g, 67% yield). ^{13}C NMR ($CDCl_3$) 143.12, 137.13, 129.9, 129.6, 127.3, 127.1 (aromatic); 69.0 (CH_2O); 53.3, 53.0, 52.3, 52.2, 49.2, 41.0 (CH_2N); 21.4 (CH_3). Anal. Calcd for $C_{70}H_{104}O_{14}N_{12}S_6$: C, 54.95; H, 6.85; N, 10.98. Found: C, 54.98; H, 6.30; N, 9.60.

1,4,7,13,16,19-Hexaaza-10,22-dioxa-1,4,7,13,16,19-hexakis(2-aminoethyl)cyclotetracosane (3). In a 250-mL flask compound **3a** (1.58 g), phenol (1.50 g), and 100 mL of 33% HBr in AcOH were stirred under reflux for 20 h. The mixture was cooled and decanted. The solid was filtered, washed with ether (200 mL), and dried under vacuum. It was dissolved in water and passed over a Dowex anion-exchange resin (OH^- form) to yield the free amine **3**, which was converted into the hydroscopic hexahydrochloride salt by addition of hydrochloric acid (0.88 g, 82% yield). ^1H NMR (D_2O) 3.68, 3.74 (br m, 48 H, CH_2N); 4.09 (br m, 8 H, CH_2O). Anal. Calcd for $C_{28}H_{80}O_2N_{12}Cl_{12}\cdot 3H_2O$: C, 31.05; H, 8.16; N, 14.32. Found: C, 31.25; H, 7.66; N, 14.32.

6,9,12-Tris(*p*-tolylsulfonyl)-6,9,12-triaza-3,15-dioxaheptadecane-1,17-diyl Bis(methanesulfonate) (13). In a 250-mL flask compound **13b**[27] (6.32 g, 8.5 mmol), Et_3N (6 mL), and dry CH_2Cl_2 (100 mL) were stirred while cooling in an ice bath. To this mixture was added dropwise over a period of 30 min a solution of methanesulfonyl chloride (1.6 mL) in dry CH_2Cl_2 (50 mL). The solution was further stirred at room temperature for another 2 h. The mixture was washed with ice water (100 mL), cold 1 N HCl (100 mL), a saturated solution of $NaHCO_3$ (100 mL), and brine (100 mL) and was dried ($MgSO_4$). Evaporation left a slightly yellow viscous liquid, which was used for the next step without further purification (7.55 g, 98.5% yield).

1,4,7,13,17,21-Hexakis(*p*-tolylsulfonyl)-1,4,7,13,17,21-hexaaza-10,24-dioxacyclohexacosane (4a). In a 1-L flask compound **13d** (5 g, 8.4 mmol) (prepared as previously described[30]), Cs_2CO_3 (27 g, 10 equiv), and DMF (300 mL) were stirred and heated to 95 °C. To this mixture was added dropwise over a period of 1 h a solution of compound **13c** (7.55 g, 8.4 mmol) in DMF (200 mL). The mixture was further stirred at 95 °C for 72 h. After the mixture was allowed to cool to room temperature, it was filtered and the solid was washed with CH_2Cl_2 (100 mL). The filtrate and washes were combined and evaporated to dryness, leaving a colored residue, which was taken up in CH_2Cl_2 (250 mL). Addition of 1 N NaOH (200 mL) caused an emulsion, which was dispersed by addition of brine. The organic layer was dried ($MgSO_4$) and the solvent removed, leaving 13.83 g of a mixture. Pure **4a** was obtained as a colorless glass after chromatography (silica gel/MeOH–CH_2Cl_2, 0–1%) (4.89 g, 44% yield). ^1H NMR ($CDCl_3$) 1.80 (m, 4 H, $CH_2CH_2CH_2$); 2.36 (s, 18 H, CH_3); 2.8–3.7 (br m, 32 H, CH_2N, CH_2O); 7.30, 7.75 (m, 24 H, aromatic). ^{13}C NMR ($CDCl_3$) 143.7, 143.5, 143.2, 136.8, 136.1, 135.6, 129.9, 129.7, 127.3, 127.2 (aromatic); 70.3, 70.2 (CH_2O); 49.7, 49.5, 49.4, 48.0, 47.6, 46.9 (CH_2N); 28.6 ($CH_2CH_2CH_2$); 21.45 (CH_3). MS *m/z* 1143 (M^+ – Ts), 989 (M^+ – 2Ts), 833 (M^+ – 3Ts), 678 (M^+ – 4Ts). Anal. Calcd for $C_{60}H_{78}O_{14}N_6S_6$: C, 55.49; H, 6.05; N, 6.46. Found: C, 55.76; H, 6.17; N, 6.75.

1,4,7,13,17,21-Hexaaza-10,24-dioxacyclohexacosane (4). In a 300-mL flask compound **4a** (4 g, 3.07 mmol), phenol (5 g), and HBr in AcOH (33%, 250 mL) were stirred and refluxed for 30 h. The mixture was cooled and ether (250 mL) was added. The precipitate was decanted, filtered, and washed with ether (250 mL). The slightly pink solid was dissolved in H_2O and passed over a Dowex (OH^- form) anion-exchange resin to yield the free amine **4**, which was converted in situ by addition of HCl to the hexahydrochloride salt. It was recrystallized from EtOH–H_2O (1.73 g, 95% yield, mp 252–254 °C). ^1H NMR (D_2O) 2.29 (m, 4 H, $CH_2CH_2CH_2$); 3.22–3.55 (m, 16 H, CH_2N); 3.65 (br m, 8 H, CH_2N); 3.92 (m, 8 H, CH_2O). ^{13}C NMR (D_2O) 68.2, 68.1 (CH_2O); 50.6, 50.2, 50.0, 47.5, 46.6, 46.5 (CH_2N); 25.1 ($CH_2CH_2CH_2$). Anal. Calcd for $C_{18}H_{48}O_2N_6Cl_6\cdot EtOH$: C, 37.57; H, 8.51; N, 13.14. Found: C, 37.92; H, 8.35; N, 13.81.

1,4,7,12,15,18-Hexakis(*p*-tolylsulfonyl)-1,4,7,12,15,18-hexaazacyclododecosane (6a). In a 1-L flask *N,N',N''*-tris(*p*-tolylsulfonyl)diethylenetriamine (6.5 g, 11.5 mol) (prepared as previously described[27]), Cs_2CO_3 (22.5 g, 68.9 mmol), and DMF (350 mL) were stirred and heated to 95 °C. To this mixture was added dropwise over a period of 1.5 h a solution of 1,15-bis(methanesulfonoxy)-5,8,11-tris(*p*-tolylsulfonyl)-5,8,11-triazapentadecane (9.94 g, 11.5 mmol) (prepared as previously described[43]) in DMF (200 mL). The mixture was further stirred and heated to 95 °C for 54 h. After the mixture was allowed to cool to room temperature, the solid was removed by filtration and the solvent was evaporated to dryness, affording a colored residue, which was taken up in CH_2Cl_2 (400 mL). Addition of water (250 mL) caused an emulsion, which was dispersed by addition of brine (200 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness, leaving 14.5 g of a mixture. Pure **6a** was obtained as a white solid after chromatography (alumina, CH_2Cl_2) (10 g, 70% yield, mp 249 °C (lit.[38] mp 245–250 °C)). ^{13}C NMR ($CDCl_3$) 143.5, 135.8, 135.7, 129.8, 127.5, 127.4 (aromatic); 49.8, 49.5, 48.5 (CH_2N); 25.6 ($CH_2CH_2CH_2$); 21.5 (CH_3). The ^1H NMR spectrum was in agreement with published data.[43]

1,4,7,12,15,18-Hexaazacyclododecosane (6). Compound **6a** (5 g, 4.03 mmol) was treated as for compound **4** (phenol, 5 g; 48 h), affording **6**-6HCl (1.67 g, 78% yield), mp >260 °C). ^1H NMR (D_2O) 2.00 (br m, 8 H, CH_2CH_2N); 3.36 (m, 8 H, CH_2CH_2N); 3.66 (br m, 16 H, CH_2N). ^{13}C NMR (D_2O) 48.6, 45.4, 44.7 (CH_2N); 23.9 ($CH_2CH_2CH_2$). Anal. Calcd for $C_{16}H_{44}N_6Cl_6\cdot H_2O$: C, 34.86; H, 8.42; N, 15.24. Found: C, 34.55; H, 9.04; N, 14.58.

1,4,7,13,16,19-Hexakis(*p*-tolylsulfonyl)-1,4,7,13,16,19-hexaazacyclotetracosane (7a). In a 500-mL flask *N,N',N''*-tris(*p*-tolylsulfonyl)diethylenetriamine (6.60 g, 11.8 mmol) (prepared as previously described[27]), Cs_2CO_3 (24 g, 70.8 mmol), and DMF (200 mL) were stirred and heated to 90 °C. To this mixture was added dropwise over a period of 2 h a solution of 1,17-bis(methanesulfonoxy)-6,9,12-tris(*p*-tolylsulfonyl)-6,9,12-triazaheptadecane (10.57 g, 11.8 mmol) (prepared as previously described[43]) in DMF (200 mL). The mixture was further stirred and heated to 90 °C for 66 h. After the mixture was allowed to cool to room temperature, the solid was filtered and the solvent evaporated to dryness, leaving a yellow residue, which was taken up in CH_2Cl_2 (300 mL). The addition of water (250 mL) caused an emulsion, which was dispersed by addition of brine (150 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness, affording 18 g of yellow solid. Pure **7a** was obtained as a white solid after chromatography on silica, eluted with $CHCl_3$. It was recrystallized by cooling from a mixture of $CHCl_3$, acetone, hexane, and DMF (9 g, 60% yield, mp 205 °C (lit.[38] mp 203–205 °C)). ^{13}C NMR ($CDCl_3$) 143.4, 135.8, 129.8, 127.4 (aromatic); 50.2, 48.8 (CH_2N); 28.4 (CH_2CH_2N); 23.6 ($CH_2CH_2CH_2$); 21.5 (CH_3). The ^1H NMR spectrum was in agreement with published data.[43]

1,4,7,13,16,19-Hexaazacyclotetracosane (7). Compound **7a** (6 g, 5.7 mmol) was treated as for compound **4** (phenol, 6 g; 72 h), affording **7**-6HCl (2.26 g, 85% yield, mp >260 °C). ^1H NMR (D_2O) 1.45–2.0 (br m, 12 H, $CH_2CH_2CH_2N$); 3.25 (m, 8 H, $CH_2CH_2CH_2N$); 3.6 (m, 16 H, CH_2N). ^{13}C NMR (D_2O) 48.9, 45.10, 44.5 (CH_2N); 26.0 (CH_2CH_2N); 23.7 ($CH_2CH_2CH_2$). Anal. Calcd for $C_{18}H_{48}N_6Cl_6$: C, 38.52; H, 8.71; N, 14.97. Found: C, 38.28; H, 8.71; N, 14.23.

1,4,7,10,13-Pentakis(*p*-tolylsulfonyl)-1,4,7,10,13-pentaazatridecane (14a). In a 3-L flask tosyl chloride (475 g, 2.5 mmol), H_2O (400 mL),

(46) Richman, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268–2270. Atkins, T. J.; Richman, J. E.; Oettle, W. J. *Org. Synth.* **1978**, *58*, 86–98.
(47) Vriesema, B. K.; Buter, J.; Kellogg, R. M. *J. Org. Chem.* **1984**, *49*, 110–113.

and diethyl ether (400 mL) were stirred and cooled to 0 °C in an ice bath. To this mixture was added dropwise over a period of 1 h a solution of tetraethylenepentaamine (94.5 g, 0.5 mmol) and NaOH (100 g, 2.5 mol) in H_2O (800 mL). Stirring was continued at room temperature for another 3 h. The yellow precipitate was filtered and washed with Et_2O and water. Pure **14a** was obtained by crystallization from hot $CHCl_3$/MeOH (230 g, 48% yield, mp 166–168 °C). ^1H NMR ($CDCl_3$) 2.40, 2.43, 2.46 (s, 15 H, CH_3); 3.18, 3.37 (br, 16 H, CH_2N); 5.56 (br, 2 H, NH); 7.30–7.39, 7.69–7.79 (m, 20 H, aromatic). Anal. Calcd for $C_{45}H_{53}O_{10}N_5S_5$: C, 53.78; H, 5.56; N, 7.29. Found: C, 53.88; H, 5.58; N, 7.48.

3,6,9,12,15-Pentakis(*p*-tolylsulfonyl)-3,6,9,12,15-pentaazaheptadecane-1,17-diol (14b). In a 500-mL flask, compound **14a** (27 g, 28.1 mmol), K_2CO_3 (38.8 g, 281 mmol), and DMF (300 mL) were stirred and heated to 95 °C. To this suspension was added dropwise over a period of 20 min, a solution of 2-bromoethanol (10 mL, 140 mmol) in DMF (50 mL). Stirring and heating were continued for another 60 h. After the mixture was allowed to cool to room temperature, the solid was removed by filtration and washed with CH_2Cl_2 (200 mL). The organic layer was evaporated to dryness, leaving a viscous liquid, which was taken up in CH_2Cl_2 (200 mL), washed with water (200 mL), and dried ($MgSO_4$). Removal of the solvent gave 30 g of a crude mixture. Pure **14b** was obtained as a white powder after chromatography (alumina/MeOH–CH_2Cl_2, 0–1%). It was recrystallized from EtOH–hexane (25.15 g, 85% yield, mp 162–164 °C). ^1H NMR ($CDCl_3$) 2.42 (s, 15 H, CH_3); 3.35 (br, 20 H, CH_2N); 3.74 (br m, 6 H, CH_2OH, OH); 7.31, 7.80 (m, 20 H, aromatic). ^{13}C NMR ($CDCl_3$) 144.1, 143.9, 143.7, 135.8, 135.4, 135.0, 130.1, 130.0, 127.6, 127.5 (aromatic); 62.0 (CH_2OH); 52.8 (CH_2CH_2OH); 49.8, 49.7, 49.6 (CH_2N); 21.6 (CH_3). Anal. Calcd for $C_{47}H_{61}O_{12}N_5S_6$: C, 53.85; H, 5.86; N, 6.68. Found: C, 52.98; H, 5.39; N, 6.47.

1,17-Bis(methanesulfonoxy)-3,6,9,12,15-pentakis(*p*-tolylsulfonyl)-3,6,9,12,15-pentaazaheptadecane (14c). In a 500-mL flask compound **14b** (15 g, 14.3 mmol), Et_3N (10 mL, 71.5 mmol), and dry CH_2Cl_2 (250 mL) were stirred and cooled in an ice bath. To this solution was added dropwise over a period of 20 min a solution of methanesulfonyl chloride (2.9 mL, 37.2 mmol) in dry CH_2Cl_2 (50 mL). The mixture was further stirred at room temperature for 1.5 h and then washed with ice water (150 mL) cold 10% HCl (150 mL), a saturated solution of $NaHCO_3$ (150 mL), and brine (150 mL). The organic layer was dried over $MgSO_4$ and the solvent removed, affording a white solid, which was dried under vacuum overnight. This compound **14c** was used in the next step without further purification (16.76 g, 97% yield, mp 176–178 °C). ^1H NMR ($CDCl_3$) 2.4 (s, 15 H, CH_3(Ts)); 3.0 (s, 6 H, CH_3(Ms)); 3.37 (br, 20 H, CH_2N); 4.39 (m, 4 H, CH_2OMs); 7.35, 7.82 (m, 20 H, aromatic). ^{13}C NMR ($CDCl_3$) 144.2, 144.05, 135.4, 135.1, 130.2, 130.1, 127.7, 127.6 (aromatic); 68.2 (CH_2OMs); 49.8, 49.5, 49.4 (CH_2N); 37.6 (CH_3CMs); 21.6 (CH_3(Ts)). Anal. Calcd for $C_{49}H_{65}O_{16}N_5S_7$: C, 48.86; H, 5.44; N, 5.81. Found: C, 48.96; H, 5.33; N, 5.71.

1,4,7,10,13,16,19,22,25,28-Decakis(*p*-tolylsulfonyl)-1,4,7,10,13,16,19,22,25,28-decaazacyclotriacontane (10a). In a 1-L flask compound **14a** (12.75 g, 13.3 mmol), Cs_2CO_3 (43 g, 133 mmol), and DMF (500 mL) were stirred and heated to 95 °C. A solution of compound **14c** (16 g, 13.3 mmol) in DMF (200 mL) was added dropwise over a period of 2 h. Stirring and heating were continued for another 48 h before the mixture was allowed to cool to room temperature. The solid was removed by filtration and washed with CH_2Cl_2 (150 mL). The organic layer was evaporated to dryness, leaving a solid residue, which was taken up in CH_2Cl_2 (100 mL) and DMF (100 mL), washed with brine (250 mL), dried ($MgSO_4$), and evaporated to dryness, leaving 20.5 g of a mixture. Pure **10a** was obtained as a white solid after chromatography (alumina/CH_2Cl_2). It was recrystallized from CH_2Cl_2–hexane or CH_2Cl_2–EtOH (8 g, 30% yield, mp >260 °C). ^1H NMR ($CDCl_3$) 2.40 (s, 30 H, CH_3); 3.30 (m, 40 H, CH_2N); 7.25, 7.75 (m, 40 H, aromatic). ^{13}C NMR ($CDCl_3$) 143.5, 135.6, 129.8, 127.5 (aromatic); 49.4 (CH_2N); 21.5 (CH_3). MS *m/z* 1971 (M^+), 1816 (M^+ – Ts). Anal. Calcd for $C_{90}H_{110}O_{20}N_{10}S_{10}$: C, 54.80; H, 5.62; N, 7.10. Found: C, 54.57; H, 5.86; N, 7.26.

1,4,7,10,13,16,19,22,25,28-Decaazacyclotriacontane (10). In a 250-mL flask compound **10a** (5.87 g, 2.97 mmol), phenol (6 g), and 33% HBr/AcOH (200 mL) were refluxed for 66 h. After the mixture was allowed to cool to room temperature, the solid was filtered, washed with ether (150 mL), and dried under vacuum. The slightly pink solid was dissolved in water and passed over a Dowex anion-exchange resin (OH^- form). The free amine **10** was collected and acidified with concentrated HCl (pH 1). The volume of the solution was reduced and the hydrochloride salt was precipitated with EtOH (1.65 g, 75% yield, mp >260 °C). ^1H NMR (D_2O) 3.46 (s, 40 H, CH_2N). Anal. Calcd for $C_{20}H_{58}N_{10}Cl_8 \cdot H_2O$: C, 32.44; H, 8.17; N, 18.92. Found: C, 32.73; H, 8.29; N, 18.16.

Isolation of the Intermediate 1-PN. A mixture of compound **1** (30 mM) and acetyl phosphate (30 mM) at pH 7 was heated to 50 °C for 15 min; it was then cooled rapidly to 25 °C and the pH was raised to 10.5. Addition of ca. 1 equiv of $BaCl_2$ precipitated all phosphate-containing compounds except the phosphorylated macrocycle **1**-PN. After the pH was raised to 14 and evaporation to dryness under vacuum without heating, the unreacted macrocycle **1** was extracted from the residue with CH_2Cl_2. The remaining white solid, which contained compound **1**-PN and acetate, was dissolved in D_2O for the NMR experiments.

Kinetic Measurements and Methods. ^{31}P NMR spectra were recorded at 81.015 MHz on a Bruker SY spectrometer; the chemical shifts are given downfield from 85% H_3PO_4 as external reference. Probe temperature was regulated by the variable-temperature accessory (±5 °C); in order to avoid heating, heteronuclear decoupling was performed at low power.

The solution pH was recorded at 25 °C using a Metrohm 636 titrimeter; adjustments of pH were made by using 5 M NaOH or HCl solutions.

Kinetic studies were performed by following the time evolution of the proton-decoupled ^{31}P FT-NMR spectra (50–500 acquisitions) of (substrate + macrocycle) mixtures. Since the ^{31}P NMR signals of acetylphosphate (AcP), pyrophosphate (PP), the intermediate phosphoramidate (PN), and phosphate (P) are distinct, the appearance or disappearance of these species could be followed simultaneously and the relative amounts obtained by signal integration (±5%). The NMR samples (2 mL in 10-mm-o.d. tubes) contained 0.03 M AcP and 0.03 M macrocycle in H_2O/D_2O 9/1 adjusted to the desired pH at 25 °C. The change in pH during the reaction was usually <0.5, which should not affect significantly the rates. The values of k_{obsd} calculated from the data were reproducible to ±10% at 40 °C and to ±20% at 84 °C.

The amounts of a given compound X present in solution have been given in the tables and figures as the percent fraction of the total phosphorus content of a sample, determined from the areas S_X (integral of signal X) and $\sum S_X$ (sum of the integrals of all signals) of the signals observed in the ^{31}P NMR spectrum: $(\%)_X = 100S_X/\sum S_X$. Since $\sum S_X$ measures total P content, $\sum S_X \propto [AcP]_i$ (initial AcP concentration), and the molar concentration of the monophosphorylated compounds AcP, P, and PN is given by $[X] = (\%)_X[AcP]_i/100$. The molar concentration of PP corresponds to half the integral of the ^{31}P signal: $[PP] = (\%)_{PP}[AcP]_i/200$, where $(\%)_{PP}$ is given as the fraction of P contained in PP: $(\%)_{PP} = 100S_{PP}/\sum S_X$. The % PP given in the tables and figures is thus double the *molar* percentage of PP present in a sample. In order to compare it to literature data about PP formation from AcP[23c] it must be divided by 2.

Acknowledgment. This work was supported by the Centre National de la Recherche Scientifique (CNRS, UA 422). We thank Dr. J. P. Kintzinger, P. Maltèse, and Dr. C. Brevard, Bruker Co., Wissembourg, for their help with the NMR measurements.