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Reaction Cascade in the Supramolecular Phosphorylation of a Bis(Guanidinium) Diol

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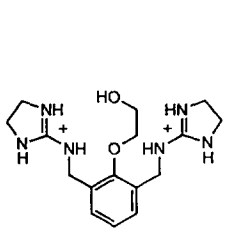
Abstract: The reaction of the bis(guanidinium) diol **4** and the cyclic phosphodiester **2** has been studied by ^{31}P NMR spectroscopy. A series of fast transphosphorylation steps was observed, each with a first order rate constant surpassing that of a control reaction by four to five orders of magnitude. These accelerations are caused by electrophilic substrate activation due to the guanidinium ions and by neighboring group effects: The substrate is held in close proximity to the hydroxy groups by ion pair formation. After phosphorylation of the 1,2-diol substructure, a rapid cyclization occurs leading to the final product **8**.

In order to mimic some of the structural and functional aspects of staphylococcal nuclease,[1,2] we recently prepared the bis(guanidinium) alcohol **1**.[3] Compound **1** forms ion pair complexes with phosphodiesters like **2**. In the presence of a base, **1** is rapidly phosphorylated producing **3**. The enormous rate increase shown by **1** compared to simple alcohols is mainly caused by the guanidinium ions; their presence leads to electrophilic activation of the phosphodiester substrates by hydrogen bond formation. Enhanced phosphorylation rates are seen with several monocationic alcohols as well.[4] Now we have modified the structure of **1** by adding a second hydroxyl group.[5] In the reaction with **2**, the bis(guanidinium) diol **4** does not form a single product. Instead, a whole cascade of fast phosphorylation and dephosphorylation steps can be observed.



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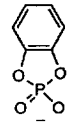

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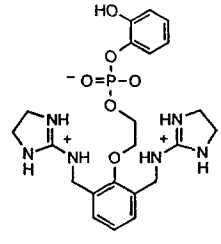

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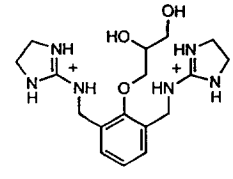

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When the reaction of **2** and **4** was monitored by ^{31}P NMR, the spectra revealed a rapid buildup and breakdown of five new signals belonging to intermediates and the formation of one stable product (0.05 M **4**, 0.15 M **2**, 0.25 M diisopropylethylamine, [D_7]DMF, 30°C). The chemical shift of the latter signal (16.71 ppm) implied the presence of a cyclic phosphate. Further evidence came from the quartet structure of the ^{1}H-coupled ^{31}P signal. Finally, the identity of **8** was confirmed by the ^{1}H NMR and ESI mass spectra [6] of an isolated

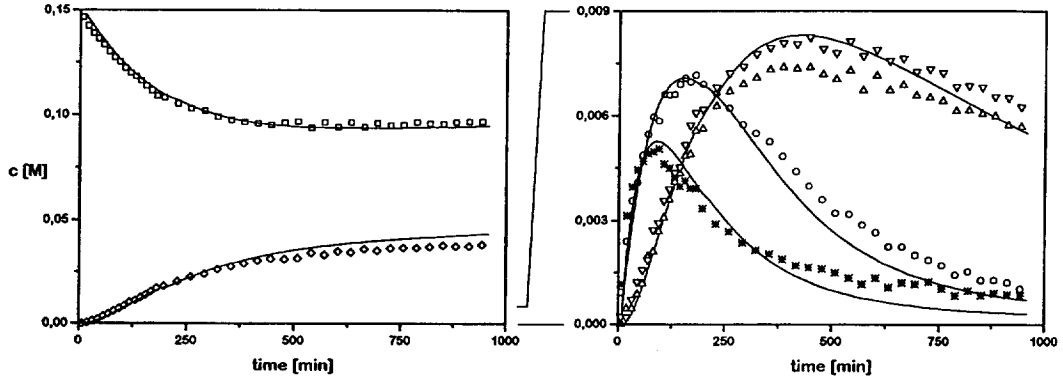


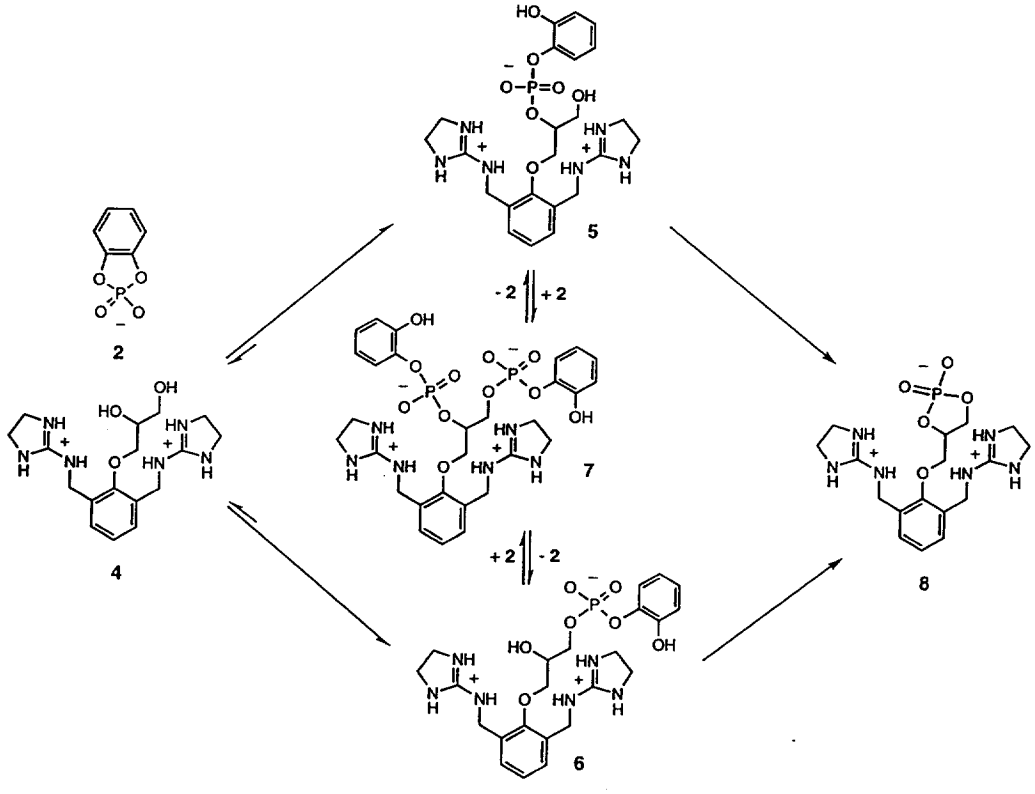
Figure 1: Concentrations of phosphorus containing compounds as a function of time (^{31}P NMR; counterions: picrate, tetramethylguanidinium). □ substrate **2** (12.57 ppm), ◊ **8** (16.71 ppm), O **5** (- 1.68 ppm), * **6** (- 0.70 ppm), ∇ **7** (*t*, - 1.11 ppm), Δ **7** (*d*, - 2.04 ppm). Calculated values are represented by continuous curves.

crystalline sample. The buildup rates of the signals at -1.68 ppm (*d*, $J_{H\text{-}P}$ = 8.7 Hz) and - 0.70 ppm (*t*, $J_{H\text{-}P}$ = 8.7 Hz) are maximal at t = 0 (Fig. 1). Consequently, these signals could be assigned to the primary phosphorylation products **5** and **6**. Within experimental error, the integrals of the remaining signals at - 1.11 ppm (*t*, $J_{H\text{-}P}$ = 8.0 Hz) and - 2.04 ppm (*d*, $J_{H\text{-}P}$ = 9.5 Hz) were identical under all conditions tested, suggesting the diphosphorylated structure **7**. As expected for a secondary reaction product, this intermediate reached its maximal formation rate later than at t = 0 (Fig. 1). Whilst the corresponding signal was visible in ESI MS, the mass spectra could not give a final proof for the existence of **7**: the signal might have been caused by a noncovalent complex of **2** and **4**. However, when experiments were run with reduced amounts of substrate **2**, the concentration of the intermediate dropped drastically. This observation is in full accord with the diphosphorylated structure **7** and the reaction scheme presented below.

By analogy with **1** (K_a = 2900 M^{-1}), **4** is expected to form stable ion pairs with phosphodiester **2**. *Quasi*-intramolecular phosphoryl transfer and production of **5** and **6** now occurs within the complex (**2** · **4**). The rates are comparable with those of compound **1**. Direct proximity between substrate and hydroxy groups and electrophilic activation by the guanidinium ions are the factors responsible for the observed high reaction rates (see below). According to molecular models, the side chains of the phosphorylation products are too short for the formation of unstrained intramolecular ion pairs, at least in the case of **5**. As a consequence, a second molecule of cyclic phosphate **2** can easily displace the phosphorylated side chains from the bis(guanidinium) units of **5** and **6**, thus preparing the second transphosphorylation step which leads to **7**. Compound **7** acts as a storage form for excess substrate **2**. As already observed in the analogous case of **3**, slow dephosphorylation of **7** later releases the compounds **5** and **6** together with formation of cyclic phosphate **2**. Finally, the thermodynamically favored compound **8** is produced by intramolecular substitution using the free hydroxy group of the glycerol side chains. After several days, **8** is the only detectable product.

In numerical simulations it is possible to obtain good agreement between calculated and experimental curves (Fig. 1). This gives further support to the scheme presented in Fig. 2 and allows the estimation of rate constants. For example, the observed first order rate constants for the buildup of **5** and **6** are $2.3 \pm 0.4 \times 10^{-3}$ min^{-1} and $3.1 \pm 0.6 \times 10^{-3}$ min^{-1}, respectively (compound **1**: 8×10^{-3} min^{-1}; conditions: 0.05 M **1** or **4**, 0.15 M **2**, 0.25 M diisopropylethylamine, [D_7]DMF, 30°C). In the presence of 0.15 M **2**, the conversion of the monophosphates **5** and **6** into **7** takes place with similar speed. Using comparable conditions, a first order rate constant of only 4×10^{-8} min^{-1} is found in the phosphorylation of the noncharged alcohol 2-phenylethanol.[1d] The final product **8** is formed predominantly by cyclization of compound **6**. For this step, a rate constant as high as 1.3×10^{-2} min^{-1} could be estimated! Here, the classical neighboring group effect of the 1,2-diol substructure is multiplied by the influence of the guanidinium ions.

The chemistry of many enzymes is characterized by a series of mechanistically related steps which are catalyzed by the same set of functional groups within the active site. Well known examples are the histidines of ribonuclease A and the oxyanion holes of serine proteases. In a similar way, each of the different phosphorylation and dephosphorylation steps seen in the reaction cascade of the bis(guanidinium) diol **4** is accelerated strongly by the proximity of the activating guanidinium ions.



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5. **4** was prepared as a racemic mixture. Selected analytical data: M.p. 198 - 199°C (MeOH/water); ^{1}H-NMR (DMSO[D_6], 270 MHz): 3.52 (*s*, 2 H, C(3')H_2), 3.62 (*s*, 8 H, ethylene-CH_2), 3.81 (*m*, 3 H, C(1')H_2 and H-C(2')), 4.45 (*d*, J = 6.0, 4 H, benzyl. CH_2), 4.84 (br., D_2O exchange, 1 H, C(3')-OH), 5.36 (br., D_2O exchange, 1 H, C(2')-OH), 7.23 (*m*, 3 H, aryl. H), 7.35 - 8.45 (br., D_2O exchange, 4 H, guanidinium-H), 8.50 (*t*, J = 6.0, D_2O exchange, 2 H, benzyl. NH), 8.60 (*s*, 4 H, picrate-H). Anal. cal. for $C_{29}H_{32}N_{12}O_{17} \cdot H_2O$ (838.66): C 41.53, H 4.09, N 20.04; found: C 41.80, H 4.37, N 20.28.
6. Selected analytical data for product cyclic phosphate **8**: ^{1}H-NMR (DMSO[D_6] + 1 % TFA[D_1], 400 MHz): 3.60 (*s*, 8 H, ethylene-CH_2), 4.05 (*m*, 2 H, C(1')H_2), 4.25 (*m*, 1 H, H-C(3')), 4.47 (*m*, 5 H, benzyl. CH_2 and H'-C(3')), 4.93 (*m*, 1 H, H-C(2')), 7.22 (*m*, 3 H, aryl. H), 8.72 (*m*, ca. 1 H, partially exchanged benzyl. NH). ^{31}P-NMR (DMSO[D_6] + 1 % TFA[D_1], 162 MHz): 16.96 (*dt*, 4 line-signal, J = 10.5 and 8.0; after ^{1}H decoupling *s*). ESI$^+$ MS (*m/z*) M^+ ($C_{17}H_{26}N_6O_5P^+$): cal.: 425.2; found: 425.1.

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