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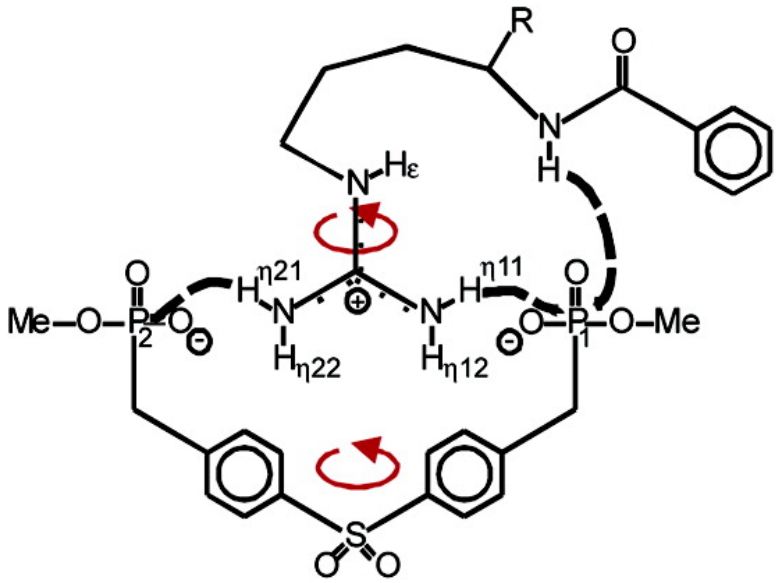

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NMR Detection of Intermolecular NH···OP Hydrogen Bonds between Guanidinium Protons and Bisposphonate Moieties in an Artificial Arginine Receptor

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Hydrogen bonds are fundamentally important in stabilizing specific intermolecular interactions. They are used to design secondary structures, to direct conformational preferences, and to control aggregation processes by nature as well as chemists. Hydrogen bonds are also particularly important in molecular recognition processes by artificial or biomolecular receptors.[1] The direct detection of scalar couplings by NMR between hydrogen donor and acceptor moiety spins in small organic complexes[2] as well as nucleic acids[2,3] and proteins[2,4] yields valuable parameters for the characterization of structures and dynamics of biological macromolecules and small organic complexes. Thus, the detection of intermolecular hydrogen bonds in artificial ligand−receptor complexes or at macromolecular interfaces would be of crucial importance. However, the direct determination of such intermolecular hydrogen bonds by NMR is still a challenge. Only a very scarce number of reports address the direct detection of hydrogen bonds at macromolecular interfaces, e.g., between amide protons and phosphate moieties[5] or between the ^{15}N spins of guanidinium groups and guanine bases.[6] By NMR, neither hydrogen bonds between guanidinium groups and phosphate moieties nor those between other acceptor moieties and individual guanidinium H_η protons have yet been directly detected. The same is also the case with hydrogen bonds within artificial ligand−receptor complexes. In contrast, in the synthetic field some artificial arginine receptors with high binding constants have been reported.[7]

Here we report the direct NMR observation of intermolecular hydrogen bonds between the guanidinium protons as well as the amide proton of α-*N*-benzoylarginine ethyl ester and the phosphorus moieties of a bisphosphonate tweezers molecule (Figure 1). The bisphosphonate tweezers were developed by T. Schrader as an artificial selective arginine receptor.[7a,d] According to molecular modeling studies,[7a] complex **1** shows a configuration of hydrogen bonding identical to those of the "arginine fork" postulated by Frankel,[8] which is a key element in RNA−protein recognition. Thus, complex **1** is an ideal synthetic model permitting the analysis of these important biological interactions.

At room temperature, the two amino groups as well as the two phosphonate groups of **1** are equivalent because of various exchange processes. Therefore, low-temperature studies were required in order to freeze out these processes. Due to solubility problems in pure CD_2Cl_2, the complex was solved in a mixture of 90% CD_2Cl_2 and 10% DMSO-d_6 and cooled to 200 K. The low-temperature spectra show separate signals for the guanidinium protons and the two phosphorus moieties (Figure 2a,d).

To detect the very small scalar couplings through hydrogen bonds from the guanidinium protons to the phosphorus moieties, one-dimensional [^{1}H,^{31}P]-HMBC spectra were recorded, a procedure

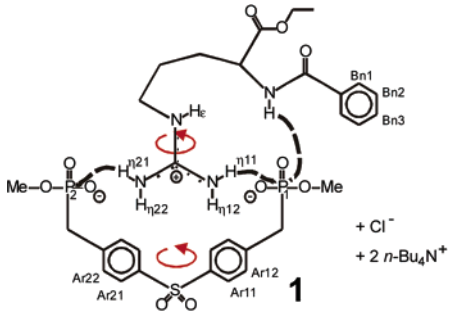

Figure 1. Complex **1** formed by a bisphosphonate tweezers molecule and α-*N*-benzoylarginine ethyl ester (rotational movements are indicated by red arrows and *trans*-hydrogen bond $^{2h}J_{HP}$ couplings by dashed lines).

that was previously used for the detection of hydrogen bonds in a Flavoprotein.[5b] To ensure that only scalar coupling is responsible for polarization transfer and that relaxation interference between ^{1}H chemical shift anisotropy and ^{1}H−^{31}P dipolar interactions (CSA/DD cross-correlation) is excluded, a modified one-dimensional [^{1}H,^{31}P]-HMBC with two simultaneously applied 180° pulses centered in the delay Δ for the buildup of ^{1}H antiphase magnetization with respect to ^{31}P was used. To further enhance the sensitivity, a partially refocused HMBC version with a selective pulse on the aromatic protons was applied (pulse sequences are given in Supporting Information). In the respective one-dimensional [^{1}H,^{31}P]-HMBC spectrum, one strong signal of the H_N proton of the amide moiety and two weak signals of the $H_{\eta 11}$ proton and the $H_{\eta 21}$ proton of the guanidinium moiety are detected, which indicate intermolecular *trans*-hydrogen bond $^{2h}J_{HP}$ couplings to the two phosphorus spins. To confirm these correlations, we applied a partially refocused one-dimensional [^{31}P,^{1}H]-INEPT with a selective pulse on the aromatic protons (contributions of CSA/DD cross-correlation were excluded as mentioned above). In the respective one-dimensional [^{31}P,^{1}H]-INEPT spectrum, the same three correlations are detected through *trans*-hydrogen bond $^{2h}J_{HP}$ couplings (Figure 2b).[9]

In the corresponding two-dimensional [^{1}H,^{31}P]-HMBC spectrum, neither could the two phosphorus signals be separated nor was a cross-peak to $H_{\eta 11}$ detected. This was due to the very short transverse relaxation times of the guanidinium protons ($T_2 \approx 15$−25 ms). Therefore, we used a partially refocused two-dimensional [^{31}P,^{1}H]-INEPT with a time-shared/constant-time chemical shift evolution[10] on phosphorus and a selective pulse on the aromatic protons (Figure 2c). In the respective two-dimensional [^{31}P,^{1}H]-INEPT spectrum (Figure 2d), all correlations expected from the one-dimensional spectra were detected ($^{2h}J_{HP} \leq 2$ Hz[11]) and the individual cross-peaks could be unambiguously assigned to the two phosphorus signals. The presence of the three cross-peaks to H_N, $H_{\eta 11}$, and $H_{\eta 21}$ clearly demonstrate the existence of $^{2h}J_{HP}$ couplings across hydrogen bonds linking the protons H_N and $H_{\eta 11}$ with phosphorus P_1 and $H_{\eta 21}$ with P_2.

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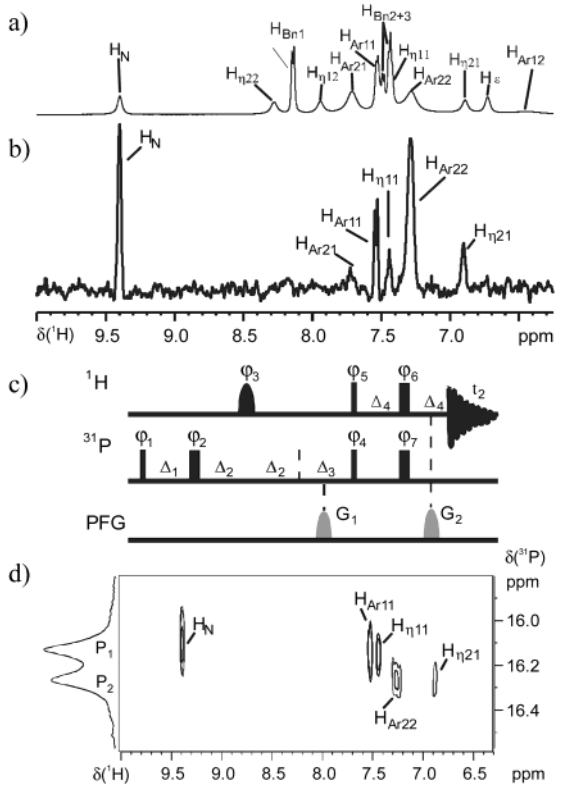

Figure 2. (a) One-dimensional ^{1}H spectrum of **1**. (b) One-dimensional [^{31}P,^{1}H]-INEPT spectrum of **1**, indicating the three *trans*-hydrogen bond correlations to H_N, $H_{\eta 11}$, and $H_{\eta 21}$ (defocusing delay 10 ms; refocusing delay 1.5 ms; NS = 7200). (c) Pulse scheme of the partially refocused two-dimensional [^{31}P,^{1}H]-INEPT with time-shared/constant-time chemical shift evolution on ^{31}P (for details, see Supporting Information). (d) Two-dimensional [^{31}P,^{1}H]-INEPT spectrum of **1**. Three cross-peaks are observed that are due to $^{2h}J_{HP}$ couplings across hydrogen bonds linking H_N and $H_{\eta 11}$ with P_1 and $H_{\eta 21}$ with P_2. All spectra were recorded at 200 K with a 50 mM sample of **1** (90% CD_2Cl_2, 10% DMSO-d_6) on a Bruker DRX 500 spectrometer.

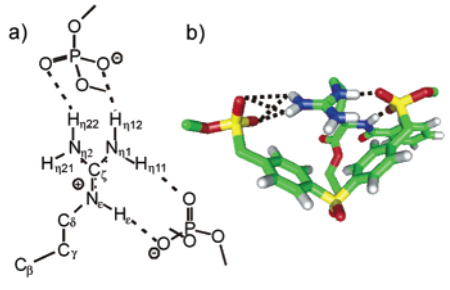

Figure 3. Asymmetrical hydrogen bonding networks with hydrogen bonds to H_ϵ proposed (a) for the arginine fork[8] and (b) by molecular dynamics calculations of **1** (hydrogen bonds are indicated by dashed lines).

The experimentally determined hydrogen bonds give clear evidence of a structure of the arginine−bisphosphonate complex formed with symmetrical end-on interactions (Figure 1). This structural arrangement deviates from the previously proposed structure of the *arginine fork*[8] (Figure 3a) as well as the structure resulting from molecular modeling studies.[7a] Both studies proposed an asymmetrical hydrogen bonding network with a hydrogen bond to the proton H_ϵ. To evaluate the influence of the organic solvent on the preferred structural arrangement, a free molecular dynamics simulation of **1** in a dichloromethane solvent box was performed. This theoretical study resulted in a complex with a side-on interaction, indicating the presence of hydrogen bonds between H_ϵ and P_1 that could not be confirmed experimentally (Figure 3b).

One of the possible explanations of the observed structural arrangement differences is the entropy factor driving the formation of small organic complexes. At room temperature, the two phosphorus signals are degenerate due to fast rotation of the tweezers relative to the arginine moiety. Even at 200 K, small cross-signals due to chemical exchange in NOESY and ROESY spectra of **1** indicate slow hindered rotation. However, to enable a symmetric rotation, all hydrogen bonds within the hydrogen bonding network established by computational methods (Figure 3) have to be broken. In contrast, in the experimentally detected symmetrical end-on interaction, concerted rotations of the tweezers molecule with the $^{\eta}NH_2$ moieties around the $N^{\epsilon}C^{\zeta}$ bond are possible. Thus, despite a remaining rotation process, the intermolecular hydrogen bonding network between the bisphosphonate moieties and the $^{\eta}$-NH_2 groups can persist. Similar end-on interactions combined with concerted rotations were found for arginine residues interacting with ligand carboxylate groups in protein ligand complexes.[12]

To conclude, we have presented the first direct NMR detection of hydrogen bonds to distinct protons of guanidinium groups. Additionally, this is the first direct NMR detection between arginine side-chain guanidinium moieties and phosphonate groups. The detected hydrogen bonding network in the investigated artificial arginine receptor shows a symmetrical end-on interaction of the guanidinium moiety, which enables concerted rotations and deviates from the structure proposed for the *arginine fork*. Together, these new methodologies provide the necessary impetus for probing more complex synthetic receptor systems to add to our current understanding of molecular recognition in synthetic versus biological receptor systems.

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Supporting Information Available: Pulse sequences, spectra, computational details, and chemical shift assignment (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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