Communication

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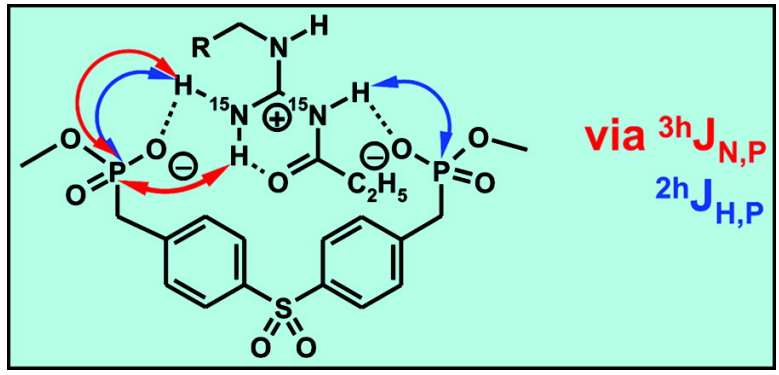

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The H-Bonding Network of Acylguanidine Complexes: Combined Intermolecular $^{2h}J_{H,P}$ and $^{3h}J_{N,P}$ Scalar Couplings Provide an Insight into the Geometric Arrangement

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Despite the crucial role of H-bonding networks in ligand recognition, enzymatic reactions, organocatalysis, and self-assembly processes, the detailed understanding of H-bonding in solution is rather limited. One reason for this is the difficulties in detecting intermolecular trans-hydrogen correlations by NMR, especially in the active center of enzymes.[1] In a remarkable study of the critical hydrogen bonds of an aminotransferase, Limbach et al. reported that the active site environment of an enzyme is better modeled using aprotic solvents than water.[2] This showed impressively the relevance of model systems in organic solvents for the understanding of enzymatic interactions. However, for the biologically so important H-bonding network of arginine side chains, only three studies on biomacromolecules[3] and our study of a synthetic arginine complex have been published so far.[4] The effect of acylation of guanidinium moieties on the binding mode and H-bond strengths has not yet been investigated, although this topic is of great relevance for applications in medicinal chemistry. Acylguanidines are successfully used in a variety of pharmacologically active compounds;[5,6] for example, in the field of G-protein coupled receptors, acylguanidines provide significantly improved pharmacokinetics, activities, and selectivities in several receptor families.[6]

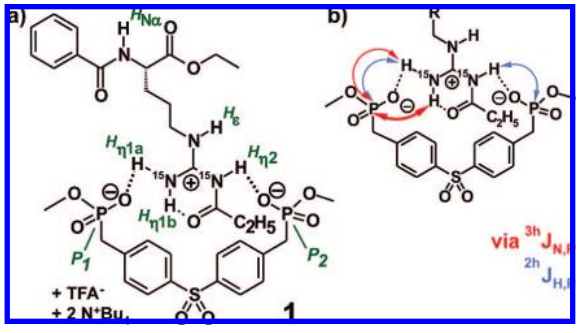

Figure 1. (a) Complex **1** formed by a bisphosphonate tweezers molecule and Bz-Arg(N^η-propionyl)-OEt (TFA = trifluoro acetic acid); (b) Detected magnetization transfers mediated by intermolecular $^{2h}J_{H,P}$ (blue arrows) and $^{3h}J_{N,P}$ (red arrows) couplings. The thicknesses of the arrows correspond to the relative intensities of the correlations.

Therefore, the H-bonding network of an acylguanidine derivative in an artificial arginine receptor[7] (see **1** in Figure 1a) was investigated. To allow for a direct comparison of acylated and nonacylated guanidines, the identical tweezers and arginine protecting groups were selected as in a previous study.[4] Unlabeled and double ^{15}N labeled arginine derivatives were used to detect 1D, 2D, and 3D correlations effected by intermolecular $^{2h}J_{H,P}$ and, for the first time in nonbiomacromolecules, by $^{3h}J_{N,P}$ couplings. In addition, the combined information of $^{2h}J_{H,P}$ and $^{3h}J_{N,P}$ coupling constants allows first experimental insights into the binding mode and geometry of the H-bonding network of an acylguanidine.

At 300 K in 90% CD_2Cl_2 and 10% DMSO-d_6, the ^1H spectrum of **1** shows separated and sharp signals of the acylguanidinium protons (see Supporting Information, Figure S2). This indicates a strong preorganization of the acylguanidinine, because in nonacylated **1**, temperatures below 230 K were necessary to detect separated ^1H guanidinium signals.[4] The ^{31}P spectrum of **1** shows only one broad signal at 300 K (see Figure S3), which indicates remaining rotational processes of the tweezers. Therefore, low temperature studies were performed to stabilize the intermolecular H-bonds. At 220 K, optimal ^1H and ^{31}P spectra were obtained (see Figure 2a,c). One-dimensional [^1H, ^{31}P]-HMBC spectra were recorded to detect cross signals caused by the very small $^{2h}J_{H,P}$ couplings in intermolecular H-bonds (see Figure 2b). In the resulting 1D [^1H, ^{31}P]-HMBC spectrum the two signals of $H_{\eta2}$ and $H_{\eta1a}$ indicate two $^{2h}J_{H,P}$ couplings between the acylguanidine and the phosphorus spins. In the 2D [^1H, ^{31}P]-HMBC spectrum (see Figure 2c), the two cross peaks $H_{\eta2}/P_2$ and $H_{\eta1a}/P_1$ identify the H-bond acceptors and reveal an end on binding mode of **1** (see Figure 1b). A coupling constant of $^{2h}J_{H\eta2,P2} = 1.3 \pm 0.4$ Hz was measured with an adapted spin−echo difference method.[8] Because of a shorter T_2-time of $H_{\eta1a}$, and severe signal overlap $^{2h}J_{H\eta1a,P1}$ could not be determined. Nonetheless, the similar signal intensities of $H_{\eta2}/P_2$ and $H_{\eta1a}/P_1$ in the 1D and 2D [^1H, ^{31}P]-HMBC suggest similar values of $^{2h}J_{H\eta2,P2}$ and $^{2h}J_{H\eta1a,P1}$.

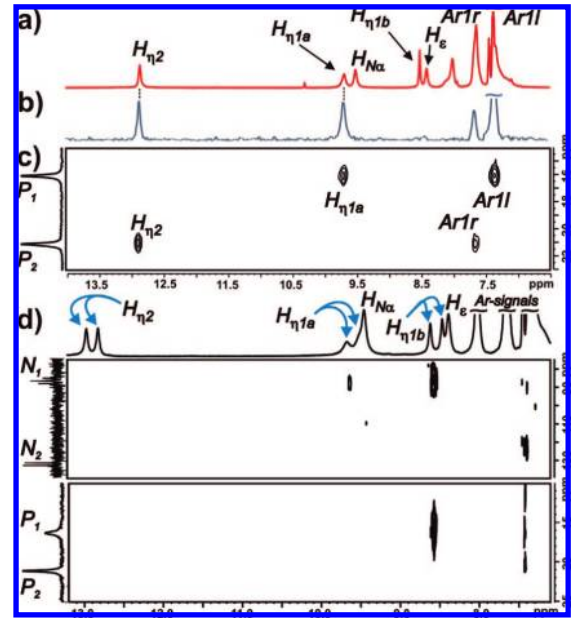

Figure 2. (a) ^1H spectrum of **1** at 220 K (90% CD_2Cl_2, 10% DMSO-d_6). (b) 1D [^1H, ^{31}P]-HMBC spectrum of **1**, indicating the two trans-hydrogen bond correlations to $H_{\eta2}$ and $H_{\eta1a}$. (c) 2D [^1H, ^{31}P]-HMBC spectrum of **1**. The two cross signals, caused by $^{2h}J_{H,P}$ couplings, connect $H_{\eta2}$ to P_2 and $H_{\eta1a}$ to P_1. (d) 2D ^1H, ^{15}N and ^1H, ^{31}P projections of a 3D HNPO spectrum of **1**. The two cross signals $H_{\eta1a}/NH_2/P_1$ and $H_{\eta1b}/NH_2/P_1$ indicate that only the $^{3h}J_{N,P}$ coupling between NH_2 and P_1 is large enough to allow for a magnetization transfer in **1**.

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The acylation leads to preorganization of the guanidine group, reduced exchange processes, larger chemical shift separations, and sharper line widths, which are interwoven factors facilitating the NMR detection of intermolecular H-bonds. This encouraged us to synthesize the double ^{15}N labeled acylguanidine[9] shown in Figure 1a to try the first magnetization transfer mediated by $^{3h}J_{N,P}$ couplings in systems other than biomacromolecules. The $^{3h}J_{N,P}$ HNPO pulse scheme applied for correlations between ^{1}H, ^{15}N, and ^{31}P of the H-bond donor and acceptor groups is conceptually similar to the 3D $^{3h}J_{N,C'}$ HNCO and $^{3h}J_{N,P}$ HNPO experiments previously used in protein complexes,[3b,10] but was adapted for small molecules in organic solvents (for pulse sequences and experimental details see Supporting Information). To optimize the HN transfer of the 3D HNPO, four 2D $^{1}H,^{15}N$ pulse sequences (with and without ^{1}H composite pulse decoupling (CPD) and sensitivity improvement, respectively) and different delay lengths for the HN correlation were tested. The resulting spectra show that a pulse sequence without CPD, with sensitivity improvement, and with a defocusing time of $(2.8\ J_{H,N})^{-1}$ is optimal. Wrong combinations reduce the intensities by up to 73%. In Figure 2d the $^{1}H,^{15}N$, and $^{1}H,^{31}P$ projections of the 3D HNPO cube are presented. The strongest cross peak is observed for $H_{\eta 1b}/NH_2/P_1$, whereas $H_{\eta 1a}/NH_2/P_1$ shows only a very weak signal and $H_{\eta 2}/NH/P_2$ is below the detection limit. Since $^{3h}J_{NH2,P1}$ is responsible for both cross peaks, their significant intensity differences reflect the different T_2-times of $H_{\eta 1b}$ and $H_{\eta 1a}$ (see Figure 2d). This effect of signal enhancement with the aid of less exchange-broadened proton signals was also reported for $^{2h}J_{N,N}$ correlations in a dynamic region of RNA.[12] In contrast, the line width of $H_{\eta 2}$ is significantly smaller than that of $H_{\eta 1a}$ and also the $2N_{x,y}H_z$ relaxation time should promote a NH/P_2 cross peak. Therefore, the missing $H_{\eta 2}/NH/P_2$ correlation indicates a significantly smaller $^{3h}J_{NH,P2}$ coupling constant compared to $^{3h}J_{NH2,P1}$.

The difficulty in detecting $^{2h}J_{H,P}$ and especially $^{3h}J_{N,P}$ correlations is well-known from protein studies. In a protein nucleotide complex, $^{2h}J_{H,P}$ and $^{3h}J_{N,P}$ coupling constants were detected for only one out of five NH···$^{-}$OP hydrogen bonds[3b] and in a flavoprotein, four $^{2h}J_{H,P}$ but only two $^{3h}J_{N,P}$ correlations were observed.[3a] On the basis of NMR studies and structural data it was concluded[3b] that an approximately linear H-bonding arrangement, expressed by the POH angle, is a prerequisite for large, detectable $^{2h}J_{H,P}$ and $^{3h}J_{N,P}$ couplings. Subsequent theoretical studies showed that both $^{2h}J_{H,P}$ and $^{3h}J_{N,P}$ probe α(POH) and the lengths of the H-bonds.[11] $^{2h}J_{H,P}$ is more sensitive to the distance r_{NO} or r_{NP} and $^{3h}J_{N,P}$ depends more on α(POH). Based on these data the relative intensities of our $^{2h}J_{H,P}$ and $^{3h}J_{N,P}$ correlations allow for the first time the derivation of information on the hydrogen-bonding geometry from combined trans-hydrogen scalar coupling in organic solvents other than freons. The detection of $^{3h}J_{NH2,P1}$ and the absence of $^{3h}J_{NH,P2}$ indicate a larger $\alpha(P_1OH_{\eta 1a})$ than $\alpha(P_2OH_{\eta 2})$. The similar values of $^{2h}J_{H\eta 2,P2}$ and $^{2h}J_{H\eta 1a,P1}$ suggest a compensation of the smaller $\alpha(P_2OH_{\eta 2})$ by a shorter distance in this H-bond. Geometry optimizations (for details see Supporting Information) of **1** support this conclusion (see Figure 3): for $NH_{\eta 1a}\cdots{}^{-}OP_1$, $\alpha(P_1OH_{\eta 1a}) = 153°$ and $r_{NP} = 4.00$ Å, and for $NH_{\eta 2}\cdots{}^{-}OP_2$, $\alpha(P_2OH_{\eta 2}) = 120°$ and $r_{NP} = 3.70$ Å. The $^{2h}J_{H\eta 2,P2}$ value is smaller than those theoretically predicted for such H-bonds. This can be explained by remaining rotations of the tweezers in **1**, because in proteins remaining dynamics of H-bonding networks can dramatically reduce the ^{h}J values.[13] A comparison with our previous study[4] shows that acylation of guanidine groups does not affect the general H-bond pattern, the end-on binding. But the acylation-induced preorganization results in up to 70 times shorter experimental times of $^{2h}J_{H,P}$ correlations, which indicates higher affinities and stronger, more covalent H-bonds.[14]

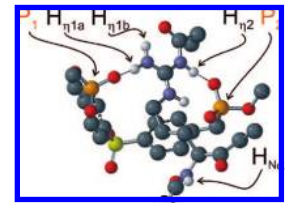

Figure 3. Spatial arrangement of the intermolecular H-bonds in **1**. The angle $\alpha(P_1OH_{\eta 1a})$ is larger than $\alpha(P_2OH_{\eta 2})$ allowing for a magnetization transfer via $^{3h}J_{N,P}$. For the sake of clarity only the NH protons are presented (for stereopicture see Figure S9 in Supporting Information).

In summary, for the first time the H-bonds of an acylguanidinium complex have been investigated, which is to our knowledge the first geometric analysis of an H-bonding network based on trans-hydrogen couplings in organic solvents other than freons. The direct NMR detection of 1D, 2D, and 3D correlations caused by $^{2h}J_{H,P}$ and $^{3h}J_{N,P}$ couplings shows an end-on binding mode in an acylguanidine bisphosphonate tweezers complex with a larger POH angle on the amine side. The acylguanidine adopts the same principal binding mode as the corresponding guanidine, but forms significantly stronger H-bonds. This may explain the success of acylguanidine ligands in medicinal chemistry applications.

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Supporting Information Available: Complete ref 5c; pulse sequences; spectra; synthetic, experimental, and computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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