Gas-Phase Reactions

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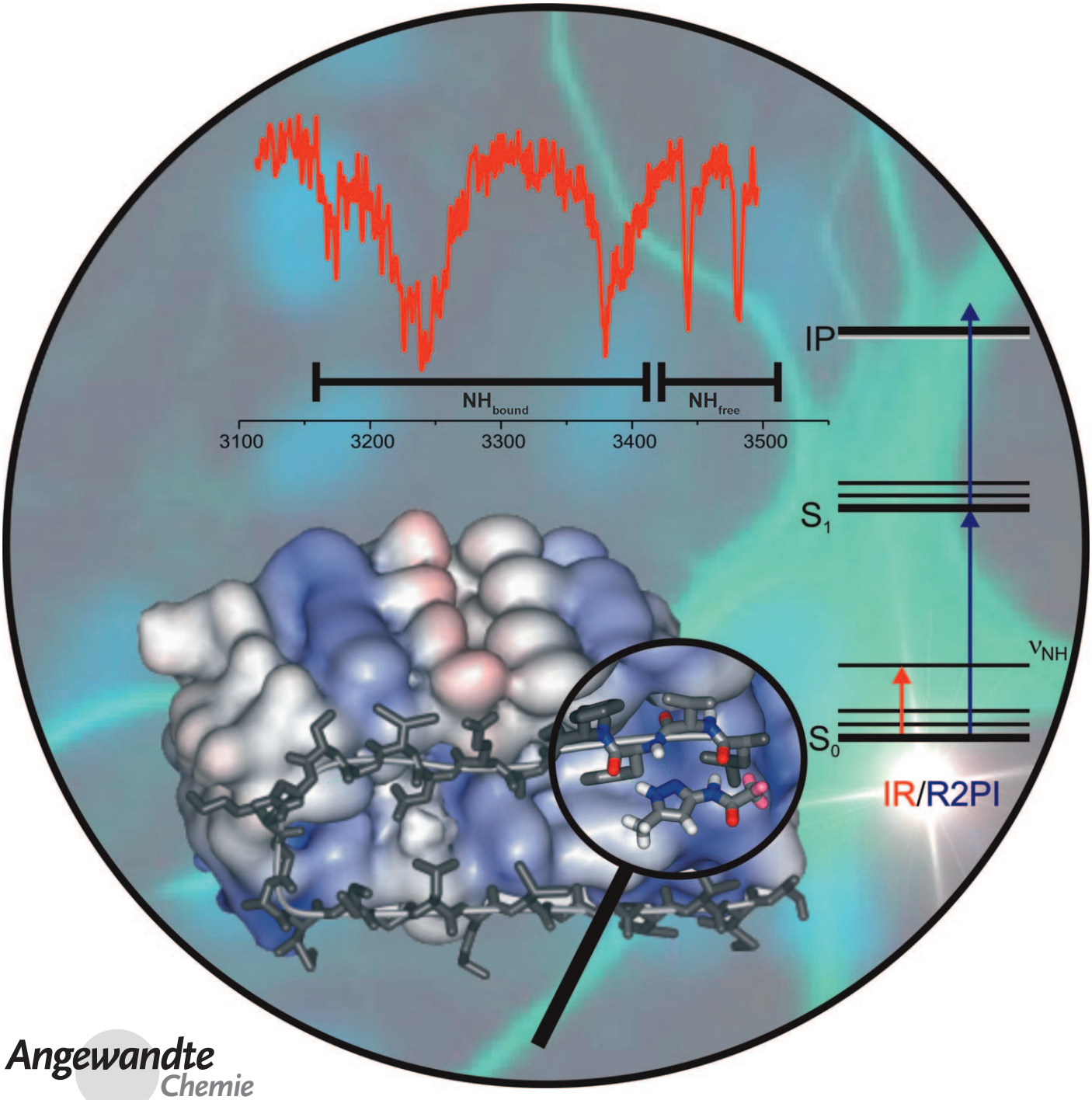
Interactions of Small Protected Peptides with Aminopyrazole Derivatives: The Efficiency of Blocking a β-Sheet Model in the Gas Phase**

*Holger Fricke, Andreas Gerlach, Claus Unterberg, Mark Wehner, Thomas Schrader, and Markus Gerhards**



In the early 1980s Prusiner and co-workers identified the misfolded prion protein (PrP^{Sc}) as a proteinacious infectious particle.[1] According to his prion model, the misfolding event itself renders the naturally occurring protein pathogenic. Specifically, its conversion from a tertiary structure rich in α helices to a structure with a high amount of β sheets is the main cause for neurological disorders such as BSE or Creutzfeldt–Jakob disease. In a related case, critical sequences (e.g., KLVFF = lysine-leucine-valine-phenylalanine-phenylalanine) in a coiled soluble peptide may form the nucleation site of a growing β sheet and eventually cause its aggregation. Thus, insoluble protein plaques, which are typical for Alzheimer's disease, are finally deposited in the brain cortex.[2] Among others, one promising strategy against protein aggregation involves the development of ligand molecules that block the solvent-exposed β sheet and terminate its growth or even dissolve existing fibrils. Kapurniotu, Meredith, et al. have pursued a similar route, which involves modification of the KLVFF sequence by N-alkylation or replacement of amide NH groups by ester oxygen atoms. These studies have led to powerful aggregation inhibitors in vitro, but for various reasons have never reached clinical trials.[3] Recently, aminopyrazole derivatives have been identified as powerful ligands with high affinity for the top face of a growing extended peptide strand. These rigid heterocyclic structures have been investigated in solution with respect to their backbone recognition abilities by various NMR and IR spectroscopic methods as well as by force-field calculations.[4–8]

To successfully compete with peptide dimerization, such a ligand must form a peptide complex with a superior gain of free enthalpy. Many different factors, however, determine its overall affinity in solution, among others solvation energies and secondary structure stabilities of the peptide environment. A method of choice for the study of intrinsic binding properties is the investigation of jet-cooled molecules or small clusters in the gas phase by spectroscopic techniques. Here, in contrast to measurements in solution, the isolated species (in our case clusters between peptides and aminopyrazoles) can be studied and their structure, dynamics, or photoreactivity are examined without any interference from a given chemical surrounding.

A great advantage of molecular beam experiments is the possibility to investigate isolated clusters mass-, isomer-, and state-selectively. The R2PI method (R2PI = resonance-enhanced two-photon ionization),[9] in which the first UV photon is used for electronic excitation and the second for ionization, allows us to distinguish between closely related isomers (e.g., tautomers), as each isomer has different electronic transitions. This is especially important if the tautomeric ligand forms distinctly different complexes with the peptides, varying in the number of hydrogen bonds (Figure 1). The formation of preferred cluster structures

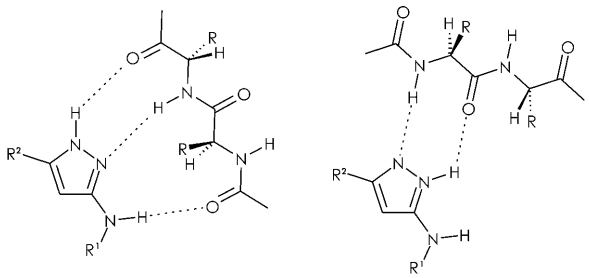


Figure 1. Binding of a peptide with normal and tautomeric MAP, highlighting the differences in hydrogen-bond donor and acceptor properties. $R^1 = H$ (MAP), $R^1 = CF_3CO$ (*tf*-MAP), $R^2 = CH_3$.

originates not only from the altered environment but also largely results from intrinsic properties of the binding partners as they optimize their hydrogen-bond strengths. In a next step, infrared spectra of the electronic ground state (S_0) can be obtained with the IR/R2PI method.[10,11] In this variant of IR/UV double resonance spectroscopy, the vibrational ground state is depopulated by absorption of an IR photon. This depopulation is detected by a decrease of the R2PI (ion) signal, which originates from the vibrational ground state. Thus a full IR spectrum of the S_0 state is obtained for each isomer (selected by the UV transitions), and overlapping spectra are circumvented. On the one hand, these IR spectra provide valuable direct information on the conformation of flexible molecules such as peptides, whose vibrational transitions are markedly different for free and hydrogen-bonded groups. On the other hand, information on relative hydrogen-bond strengths is obtained from the corresponding IR frequency shifts.

To obtain information on the selectivity and efficiency of pyrazole-based ligand molecules, we systematically studied aggregates with small model peptides. Their composition is drawn from the amino acid sequence KLVFF, which is the internal hydrophobic element of the Alzheimer's peptide responsible for aggregation nucleation. The dipeptide Ac-Val-Phe-OMe[12] and the tripeptide model Ac-Val-Tyr(Me)-NHMe[13] were already characterized in the molecular beam as monomers.[12,13] These are now combined with 5-methyl-3-aminopyrazole (MAP) and 5-methyl-3-trifluoroacetylpyrazole (*tf*-MAP). In contrast to our preliminary work on aggregates of protected amino acids with pyrazole and MAP,[14] the above-mentioned ligand–peptide pairs are now able to form the maximum number of three intermolecular hydrogen bonds. Specifically, both MAP and *tf*-MAP can form a triple donor–acceptor–donor hydrogen bond to the amide groups of the extended peptide strand (Figure 1).

[*] Dr. H. Fricke, Dr. A. Gerlach, Dr. C. Unterberg, Prof. Dr. M. Gerhards
Physikalische und Theoretische Chemie, TU Kaiserslautern
and Research Center OPTIMAS
Erwin-Schrödinger-Strasse 52, 67633 Kaiserslautern (Germany)
Fax: (+49) 631-205-2750
E-mail: gerhards@chemie.uni-kl.de
Dr. M. Wehner, Prof. Dr. T. Schrader
Institut für Organische Chemie, Universität Duisburg-Essen
Universitätsstrasse 5, 45117 Essen (Germany)

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As a first example we investigated the aggregate formed between the dipeptide Ac-Val-Phe-OMe and MAP. The corresponding IR/R2PI spectrum of the one observed isomer is shown in Figure 2a. The spectrum is recorded via the most intense transition in the R2PI spectrum at 38030 cm^{-1}. Typically, IR spectra can be divided into two regions, with NH stretching frequencies above 3400 cm^{-1} (usually free NH groups) and below 3400 cm^{-1} (usually hydrogen-bonded NH groups). All NH stretching vibrations of isolated MAP and Ac-Val-Phe-OMe appear above 3400 cm^{-1}.[12,14,15] Interestingly, the IR/R2PI spectrum of Ac-Val-Phe-OMe/MAP clearly indicates that in the cluster two NH groups are still free (3481 and 3443 cm^{-1}), whereas three other NH groups are assumed to be hydrogen-bonded (3395, 3378, and 3243 cm^{-1}) and thus potentially lead to a triply hydrogen-bonded arrangement. (The transition at 3175 cm^{-1} can be assigned to an overtone of a NH bending vibration; see also transitions at 3150 and 3152 in Figures 3 and 4.) The most intense transition at 3243 cm^{-1} can be well correlated with the NH stretching mode of hydrogen-bonded phenylalanine as obtained from our investigations on clusters of Ac-Phe-OMe with pyrazole or MAP.[14] Likewise, the NH stretching band at 3481 cm^{-1} is correlated with the asymmetric (free) NH stretching vibration of the NH_2 group in MAP.[14] The frequency of the second free NH group at 3443 cm^{-1} is in good agreement with the NH stretching vibration of the valine residue; in isolated Ac-Val-Phe-OMe, which has a β-sheet-related structure, this vibration is located at 3441 cm^{-1}. The detailed considerations above indeed suggest a triply hydrogen-bonded structure, as indicated in Figure 2.

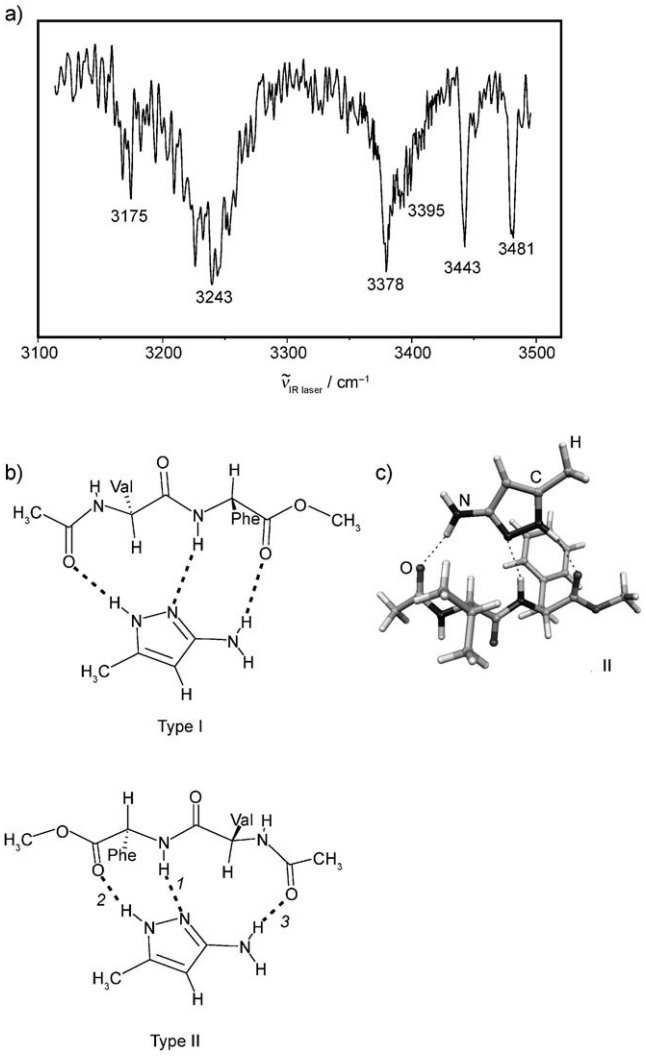

Figure 2. a) IR/R2PI Spectrum of Ac-Val-Phe-OMe/MAP (3100–3500 cm^{-1}); b) corresponding binding schemes; c) assigned structure (type II), calculated at the B3LYP/6-31+G(d) level.

To verify this assignment, Hartree–Fock and DFT geometry optimization were performed for a very large number of structures derived from force-field calculations, and subsequent normal mode analyses were carried out. Due to its good error compensation, the 3-21G(d) basis set was used in HF calculations, whereas DFT calculations were performed with the now well-established B3LYP functional in combination with a 6-31+G(d) basis set. These calculations support the assumption of a triply hydrogen-bonded arrangement for Ac-Val-Phe-OMe/MAP. However, close inspection shows that in the complex, both molecules can adopt two alternative relative orientations, which can be interconverted by simply twisting one of the molecules by 180° (in Figure 2b the peptide is rotated). DFT and single-point MP2 calculations give only an estimation of relative energies for structures that are close in energy, but an assignment can be obtained using the frequencies. Gratifyingly, the frequency calculations clearly prefer the type II structure, allowing an unambiguous structural assignment. It is interesting to note that the conformation of the isolated Ac-Val-Phe-OMe molecule is almost entirely conserved in its cluster with MAP. From the observed differences in NH stretching frequency between the isolated monomers (peptide and MAP) and the cluster, it can be concluded that only two hydrogen bonds are strong ($\Delta\tilde{\nu}_{Phe} = 208\ cm^{-1}$ and $\Delta\tilde{\nu}_{Pyr} = 129\ cm^{-1}$, with $\tilde{\nu}_{NH\text{-}Phe}(1) = 3243\ cm^{-1}$ and $\tilde{\nu}_{NH\text{-}Pyr}(2) = 3395\ cm^{-1}$) whereas the third hydrogen bond between MAP's less acidic NH_2 group and the dipeptide's acetyl group is much weaker ($\Delta\tilde{\nu}_{NH_2\text{-}bound} = 24\ cm^{-1}$ with $\tilde{\nu}_{NH_2\text{-}bound}(3) = 3378\ cm^{-1}$). This bond may be broken in favor of a stronger binding partner—an effect which would reduce the efficiency of the MAP ligand.

In the next step, we proceeded to cluster formation between MAP and Ac-Val-Tyr(Me)-NHMe, which can be regarded as a tripeptide model,[13] as it contains three full amide groups and can thus serve as a better model for a native peptide backbone conformation. Consequently, the peptide binding partner is able to form additional intramolecular hydrogen bonds, which may compete with the intermolecular binding mode to MAP. Experimentally, again only one isomer of the cluster is observed. Its IR/R2PI spectrum recorded via the electronic origin at 35648 cm^{-1} is shown in Figure 3. This spectrum looks different from that of Ac-Val-Phe-OMe/MAP, but the transitions at 3402 and 3486 cm^{-1} are identical to those observed for a free non-hydrogen-bonded NH_2 group in MAP.[14] Furthermore, the transition at 3368 cm^{-1} is in excellent agreement with the value obtained for an intramolecular N–H···O=C hydrogen bond (e.g. in Ac-Phe-NHMe[16]), forming a seven-membered ring called a γ-turn.

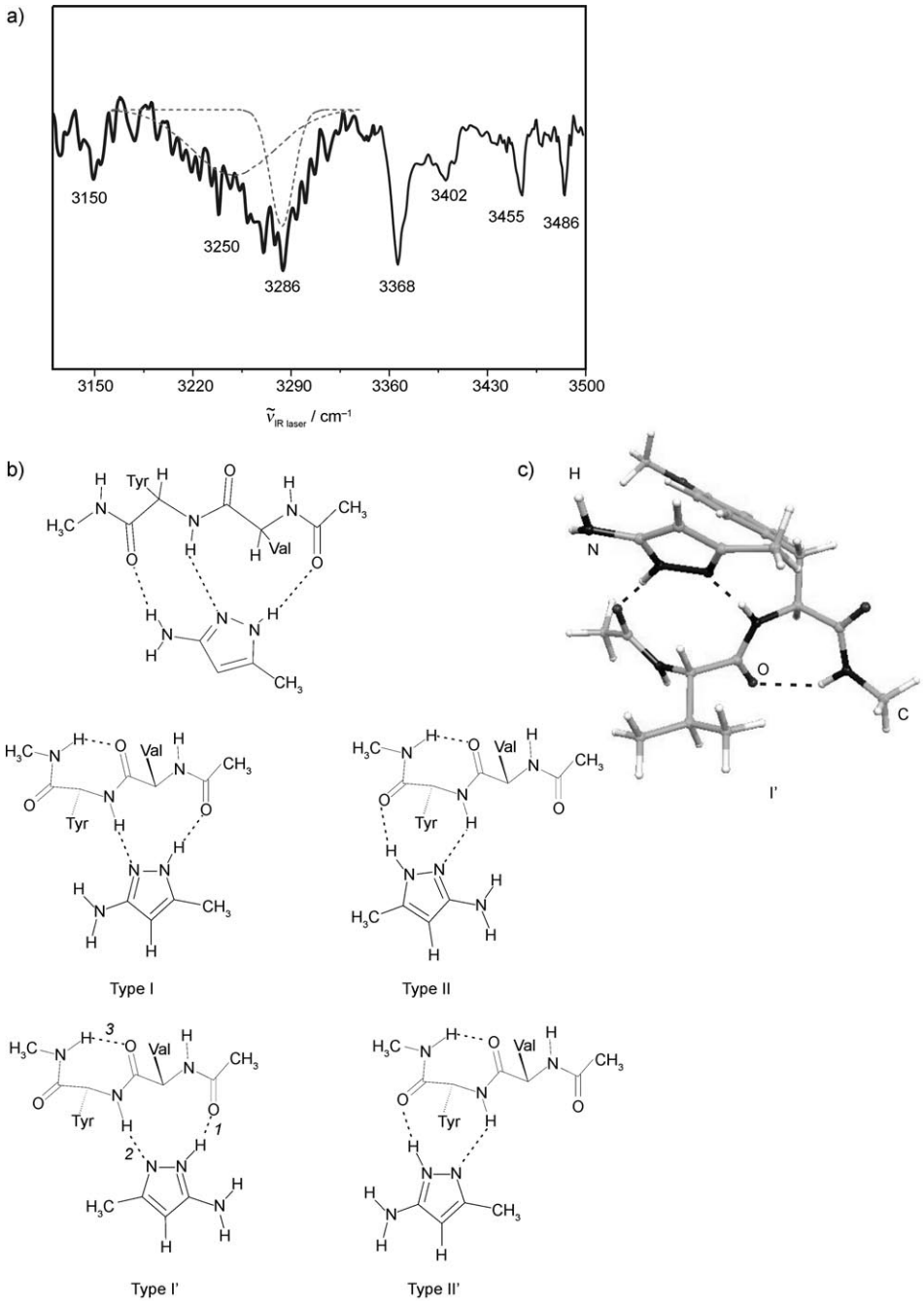

Figure 3. a) IR/R2PI spectrum of Ac-Val-Tyr(Me)-NHMe/MAP (3100–3500 cm^{-1}); b) corresponding binding schemes; c) assigned structure (type I′), calculated at the B3LYP/6-31+G(d) level.

These results indicate that now the NH_2 group of MAP is free and only two intermolecular hydrogen bonds are formed to the peptide. However, the loss of the third intermolecular hydrogen bond is effectively compensated by an additional intramolecular hydrogen bond. These results are again strongly supported by ab initio and DFT calculations as described above. Four possible structural motifs with the described combination of two inter- and one intramolecular hydrogen bond are shown in Figure 3b. By far the best agreement was found for structure I′. According to calculation and experiment, this bonding scheme comprises three strong hydrogen bonds represented by the following shifts with respect to the free NH vibrations of the monomer: $\Delta\tilde{\nu}_{Pyr} = 238$ cm^{-1}, $\Delta\tilde{\nu}_{Tyr} = 161$ cm^{-1}, and $\Delta\tilde{\nu}_{NHMe} = 111$ cm^{-1} (with $\tilde{\nu}_{NH\text{-}Pyr}(1) = 3286$ cm^{-1}, $\tilde{\nu}_{NH\text{-}Tyr}(2) = 3250$ cm^{-1}, $\tilde{\nu}_{NHMe}(3) = 3368$ cm^{-1}). Obviously, the hydrogen-bond donor properties of the MAP amino group are only weak, and if, as in the case for Ac-Val-Tyr(Me)-NHMe, a competitive stronger intramolecular hydrogen bond can be formed, it is preferred. This reorganization causes a change of the peptide conformation with respect to the monomer.

To reconstitute the triple intermolecular hydrogen-bond pattern with Ac-Val-Tyr(Me)-NHMe, the MAP amino group was trifluoroacetylated (*tf*-MAP), causing a significant rise in NH acidity. Now the IR/R2PI spectrum of Ac-Val-Tyr(Me)-NHMe with *tf*-MAP (recorded via the electronic origin at 35 524 cm^{-1}, Figure 4) exhibits only one transition (at 3465 cm^{-1}) clearly corresponding to a free NH stretching vibration. All the other NH groups are assumed to be involved in hydrogen bonds. Ab initio and DFT calculations suggest a binding motif very similar to Ac-Val-Phe-OMe/MAP, with one notable exception: In addition to the three expected intermolecular hydrogen bonds between peptide and ligand, the C-terminal NHMe group is now intramolecularly hydrogen bonded as well (Figure 4). Between the two possible relative orientations of the two analytes, ab initio and DFT calculations again prefer type II (Figure 2). This time, all intermolecular hydrogen bonds are very strong and display large frequency shifts: $\Delta\tilde{\nu}_{Pyr} = 256$ cm^{-1}, $\Delta\tilde{\nu}_{Amide} \approx 220$ cm^{-1}, $\Delta\tilde{\nu}_{Tyr} = 205$ cm^{-1}, and $\Delta\tilde{\nu}_{NHMe} = 80$ cm^{-1} (with $\tilde{\nu}_{NH\text{-}Pyr}(1) = 3268$ cm^{-1}, $\tilde{\nu}_{NH\text{-}Amide}(2) = 3238$ cm^{-1}, $\tilde{\nu}_{NH\text{-}Tyr}(3) = 3206$ cm^{-1}, and $\tilde{\nu}_{NHMe}(4) = 3397$ cm^{-1}). As desired, the strongly NH-acidified *tf*-MAP restores the triple hydrogen-bond motif. Moreover, the exchange of a C-terminal ester for an amide moiety allows formation of an extra intramolecular hydrogen bond, stabilizing the whole

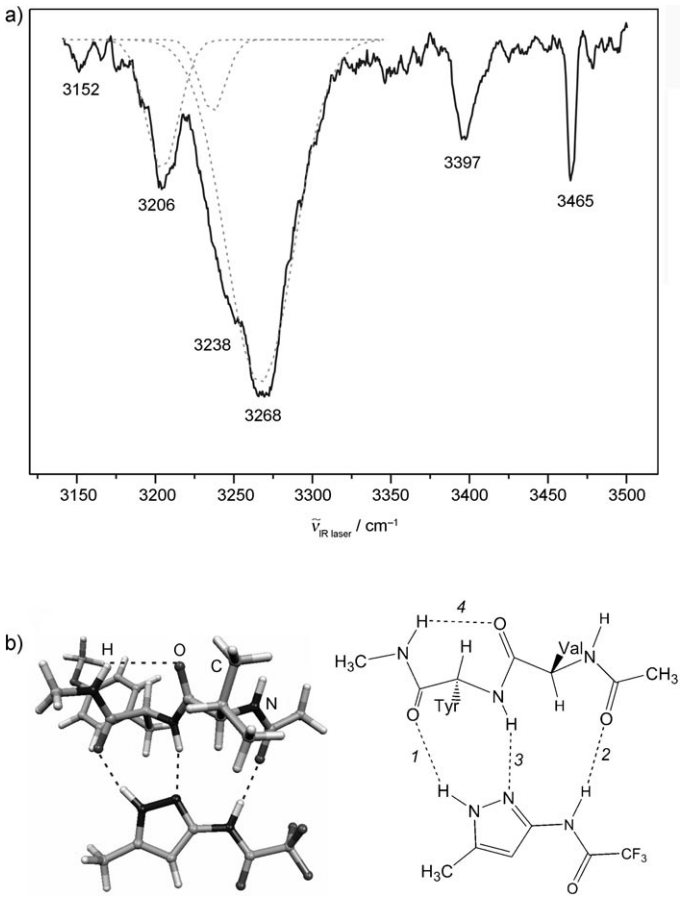

Figure 4. a) IR/R2PI spectrum of Ac-Val-Tyr(Me)-NHMe/*tf*-MAP; b) assigned structure (type II), calculated at the 6-31 + G(d) level.

aggregate. Intriguingly, the strongest hydrogen bonds are formed to the pyrazole heterocycle, a factor that may contribute to its efficiency in preventing peptide aggregation. Related studies of isolated nucleic base pairs have revealed an exceptional stability of the NH–N bond.[17] With our methods, the relative strengths of individual intermolecular hydrogen bonds in the isolated complexes can be determined. These investigations support the results of independent NMR spectroscopic studies in chloroform solution and in freon mixtures,[5,8] which indicate the aggregation of aminopyrazoles to β sheets.

We conclude that in the gas phase, isolated clusters between small peptides and designed β-sheet ligands can be studied in detail by means of highly selective spectroscopic methods (R2PI, IR/R2PI). In each case of our study, only one well-defined aggregate was formed. By combining experimental stretching vibrational frequencies with DFT and ab initio calculations, a full assignment was achieved, and the preferred peptide conformation and its relative orientation towards the ligand were determined. Specifically, the critical competition between inter- and intramolecular hydrogen bonding as well as the different strengths of all hydrogen bonds could be evaluated. The chosen strategy thus offers a valuable tool to gain deeper insight into the intrinsic properties of hydrogen-bonded peptide complexes and helps to optimize ligand design.

Experimental Section

The experimental setup has been described elsewhere,[12,13] and thus only a short description is given: The R2PI and IR/R2PI spectra were measured in a vacuum system consisting of a differentially pumped linear time-of-flight mass spectrometer and a pulsed valve (General Valve Iota One, 500 μm orifice) for skimmed jet expansion (X/D = 130). A frequency-doubled dye laser (Lumonics HD 300), pumped by a Nd:YAG laser (Lumonics HY 750), was used for excitation to the S_1 state and for ionization. The IR light in the region of 2.86–3.23 μm (3100–3500 cm^{-1}) was generated with a $LiNbO_3$ crystal by difference frequency mixing of the fundamental (1064 nm) of a seeded Nd:YAG laser (Spectra-Physics PRO-230) and the output of a dye laser (Sirah, Precision Scan) pumped by the second harmonic (532 nm) of the same Nd:YAG laser. The IR output is amplified by an optical parametric amplification (in a $LiNbO_3$ crystal) of the output of the IR laser (2.86–3.23 μm) and the fundamental of the Nd:YAG laser. Since the time delay chosen for the two lasers is not longer than 100 ns, all lasers have been spatially overlapped. To obtain IR/R2PI spectra the IR laser is fired 60 ns prior to the UV laser. The substances and the valve are differently heated with an increasing temperature gradient in the order ligand, peptide, and valve. The ligands are heated up to 110 (MAP) and 150 °C (*tf*-MAP), the peptides and valve to 160 and 170 °C. Helium was used as carrier gas (2000 mbar).

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