Gas-phase studies of small biomolecules\textsuperscript{1–3} and peptides\textsuperscript{4–9} have gained a considerable interest in the recent years as they provide experimental counterparts to the numerous high-level calculations on short peptide chains,\textsuperscript{10–13} documenting the competition between secondary structures such as $\beta$-strands, $\beta$-turns, $\alpha_2$-ribbons, $\alpha$- and $3_{10}$ helices. In the present communication, we report an optical spectroscopic study in the gas phase on the three-residue peptide chain, Ac-Phe-Gly-NH$_2$, (Ac = N-acetyl). The conformational analysis carried out from the IR spectroscopy is backed by high-level theoretical calculations, which enables a structural assignment in terms of intramolecular H-bonding. In particular, we provide evidence for the spontaneous formation of successive entangled $\beta$-turns in the gas phase.

In the experiment, molecules are laser-desorbed and cooled in a supersonic expansion.\textsuperscript{16,17} UV spectra (Figure S1) are recorded in the absorption region of the phenylalanine UV chromophore using mass-selective resonant two-photon ionization (R2PI). At least five conformers, distinguishable in the UV spectrum, are populated in the jet (labeled A1–A4 and B in Figure S1).

The IR spectrum of each conformer in the amide A region (3 $\mu$m) has been obtained in Saclay by the IR/UV double resonance technique\textsuperscript{1,2} using an OPO (1 cm\textsuperscript{-1} resolution) as an IR source.\textsuperscript{8} The IR spectra (Figure 1a) of the five conformers A1–A4 and B of Ac-Phe-Gly-NH$_2$ show resolved absorption bands assigned to NH stretching modes of the molecule. Some bands are intense, broad, and significantly red-shifted compared to the range of free or nearly free NH’s (3420–3550 cm\textsuperscript{-1}) \textsuperscript{9}. Those characteristics are unambiguous signatures of intramolecular H-bonding. The presence of IR bands in an intermediate region (3420–3450 cm\textsuperscript{-1}) indicates weaker interactions of the corresponding NH groups, as for example in $\alpha$ close contacts in $\beta$ strand-like ($\beta_L$) conformations or in NH-aromatic interactions.\textsuperscript{5,6,8} These basic considerations indicate that conformers A1–A4 in Figure 1 contain strong intramolecular H-bonds that involve at least two NH moieties. The spectrum of conformer B is qualitatively different. It exhibits two narrow and intense bands that are moderately red-shifted (at 3380 and 3392 cm\textsuperscript{-1}), indicating that two medium-strength H-bonds are present. The amide I–II (5–8 $\mu$m) absorption spectrum of this conformer (Figure 2) was recorded using the same IR/UV double resonance technique, employing the free electron laser FELIX\textsuperscript{17,18} (15 cm\textsuperscript{-1} resolution) as the IR source. In contrast to the amide A region, the amide I region exhibits only one partially resolved feature.

The here proposed H-bonding assignment of these five conformers is consistent with recently reported IR spectra of shorter peptides.\textsuperscript{19} The main conformer of Ac-Gly-Phe-NH$_2$, assigned to a

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure1}
\caption{(a) IR/UV double resonance spectrum in the amide A region of five conformers of Ac-Phe-Gly-NH$_2$ labeled A1–A4 and B. The spectral gap in the 3470–3500 cm\textsuperscript{-1} region is due to an undesired absorption in the crystal of the IR source. (b) H-bonding schemes in a series of conformations exhibiting two H-bonds (labeled 1–4), three H-bonds (5) or none (6). (c) DFT B3LYP/6-31+G(d) calculated stick spectra (scaling factor 0.960) of selected conformations (see text and Table S1) for comparison with the spectrum of conformer B.}
\end{figure}
a set of selected conformations that exhibit only two H-bonds (Figure 1b; Table S1). Considered were structures with (1) two C$_{10}$ bonds (successive entangled $\beta$-turns), (2) one C$_{10}$ and one C$_{7}$ bond (successive entangled $\beta$-turns and $\gamma$-turns in C$_{10}$–C$_{7}$ as well as C$_{7}$–C$_{10}$ order), (3) one central C$_{7}$ $\gamma$-turn accompanied by either a parallel C$_{11}$ or an antiparallel C$_{13}$ bond, and (4) having a $\beta$ strand-like ($\beta_1$; C$_{4}$) conformation on Phe (very stable in the protected amino acid$^{8,9}$) followed by two successive $\gamma$-folds (C$_{7}$–C$_{7}$). For comparison, two remarkable secondary structures have also been considered (5) a triple C$_{7}$ structure (2$\beta$ ribbon) and (6) a triple C$_{5}$ structure ($\beta$-strand). The orientation (anti, gauche+, and gauche$-$) of the Phe side-chain relative to the backbone was chosen either arbitrarily or, when possible, to allow stabilizing NH$-$H interactions.

In structures (1–2), among the four canonical types of $\beta$-turns, types I or II were chosen for the Phe-Gly part, as they have been shown to be the most stable forms in Ac-Phe-Gly-NH$_2$.$^{10}$ For the Gly-Gly part, all the four types were a priori considered, but steric considerations led to retain only I$\prime$–I and I$\prime$–II$\prime$ combinations. 3$_{10}$ helices (which also belong to family 1) were found to converge to I$\prime$–I$\prime$ forms. For $\gamma$-turns on Gly residue (families 2 and 5), both inverse or direct, i.e., $\gamma_1$ or $\gamma_2$, respectively, were considered, in contrast to $\gamma$-turns on Phe for which $\gamma_1$ are much more stable.$^8$ Figure 1c displays calculated absorption spectra in the amide A region for selected conformations characteristic of the six families described above. A large variety of red-shifts and hence of H-bond strengths is found, and the calculated spectra can be compared to the experimental absorption pattern of conformer B. This comparison (Figure 1c and Table S2) suggests that the central $\beta$-turn of the Phe-Gly part of the chain. The absence of the 3$_{10}$ helix conformation should probably be viewed as a specific effect of the Gly residue. This result might be of importance since the Gly residue, which is less demanding in terms of calculation facility, is generally considered as a case study and a popular model in theoretical studies.

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Supporting Information Available: UV spectrum of Ac-Phe-Gly-NH$_2$ (Figure S1), structural and energetic parameters (Table S1), calculated IR data (Table S2), and relevant DFT optimized structures (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

References