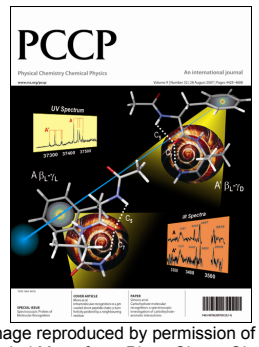

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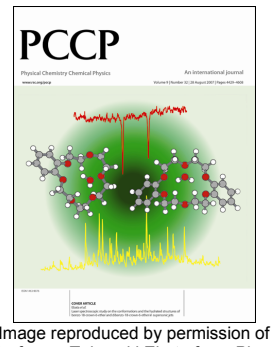

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UV resonance Raman spectroscopic monitoring of supramolecular complex formation: peptide recognition in aqueous solution
Carsten Schmuck *et al.*, *Phys. Chem. Chem. Phys.*, 2007, **9**, 4598 (**DOI:** 10.1039/b709142g)

Secondary structure binding motifs of the jet cooled tetrapeptide model Ac–Leu–Val–Tyr(Me)–NHMe

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In this paper the structure of the isolated tetrapeptide model Ac–Leu–Val–Tyr(Me)–NHMe (Leu = leucine, Val = valine, Tyr = tyrosine) is investigated by mass- and isomer-selective IR/UV double resonance spectroscopy. Two isomers of this peptide are observed and in combination with force field, *ab initio*, and DFT calculations these structures are assigned to folded arrangements presenting two different secondary structure binding motifs: (a) a combined γ-turn/β-turn structure and (b) a triple γ-turn structure, which is described for the first time for an isolated model system in the gas phase.

I. Introduction

Flexible biomolecules like peptides and proteins can occur in many different conformations. The influences leading to certain types of secondary structures can be divided into the groups of intra- and intermolecular interactions. In the case of peptides the sequence, chain length and kind of termini are examples for the intrinsic properties that determine the structure. In order to analyze these properties without the influence of an environment molecular beam experiments on isolated species can be used as a starting point. If the peptide contains one of the three aromatic amino acids (phenylalanine, tryptophan, tyrosine) resonant two photon ionization (R2PI) and fluorescence spectroscopy have been shown to be excellent tools in analyzing species in the gas phase.[1–3] Since vibrational frequencies are sensitive for different back-bone orientations of peptides, direct structural information can be obtained by recording IR spectra. Thus IR/UV double resonance spectroscopy and especially the IR/R2PI (infrared/resonant 2-photon ionization)[4] method is very powerful for determining and discriminating between different structures because it is mass- and isomer-selective.

Nowadays there are several IR data available on neutral protected amino acids,[5–9,16] dipeptides[7,10–15,17,18] and tripeptides or tripeptide models.[11,12,15,19–22,28] All of the aforementioned investigated species except for those in ref. 16 and 28 contain one of the aromatic amino acids phenylalanine,[6–8,10,13–15,17–22] tryptophan[5,9,11,15] or tyrosine.[12,22] In our publications[12,22] on the tripeptide model Ac–Val–Tyr(Me)–NHMe we assigned its structure to a β-sheet related arrangement but a β-turn structure could not be excluded. The question arises what will happen if the peptide is elongated by another amino acid and if it is possible to make an unambiguous assignment to a certain structure. The tetrapeptide model offers several structural arrangements including β-sheet related structures, β-turns, γ-turns as well as combined structural arrangements like β-turn/γ-turn conformations.

In the case of the three residue system of two alanines and one phenylalanine for all possible sequences (Ac–Ala–Ala–Phe-NH_2, Ac–Phe–Ala–Ala-NH_2 and Ac–Ala–Phe–Ala-NH_2) only one main conformer[20] is observed which always contains structural elements of the corresponding smaller system Ac–Ala–Phe–NH_2 and Ac–Phe–Ala–NH_2.[14] A combined γ-turn/β-turn, a combined β_L/γ-turn/γ-turn structure or the combination of β-turns describing a model for a 3_{10} helix are assigned. For the tripeptides Trp–Gly–Gly[11,15] and Phe–Gly–Gly[21] two and four conformers have been found with similar structures for the global minima of both species. These structures are assigned to a folded isomer where the unprotected C- and N-termini are nearby. In the case of Trp–Gly–Gly the second observed isomer has a stretched backbone with a free carboxylic group but the NH_2 group is folded back to form a hydrogen bond. This behaviour is typical for polar end groups.

A polypeptide that is protected at both termini like Ac–Leu–Val–Tyr(Me)–NHMe (*cf.* Fig. 1) could serve as a model for a small part of a protein where certain compounds in a strand are not influenced by the polar end groups. Therefore we have extended our investigations on Ac–Val–Tyr(Me)–NHMe in adding the amino acid leucine to form the tetrapeptide model Ac–Leu–Val–Tyr(Me)–NHMe. Although leucine is part of several β-sheet arrangements it also acts as a strong helix former. So it is very interesting to investigate the influence of this residue on the rest of the peptide. To our knowledge this is the first investigation on a tripeptide with three different residues by applying IR/R2PI spectroscopy. The chosen system will also be of interest since the sequence Leu–Val–Tyr is similar to the LVF (leucine valine phenylalanine) sequence of KLVFF which is part of β-sheets that are considered to be important in the formation of Alzheimer's disease. Here the KLVFF peptide sequence is involved in a process of molecular recognition. Due to the larger absorption cross section of tyrosine we

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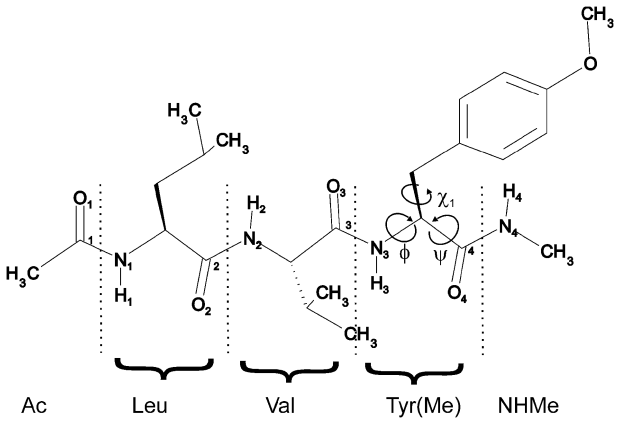

Fig. 1 Schematic picture of the tetrapeptide model Ac–Leu–Val–Tyr(Me)–NHMe. The angles describing the backbone (ϕ and ψ) and part of the side-chain (χ_1) are given for the Tyr residue.

choose the sequence LVY instead of LVF. Tyr(Me) deviates from Phe only by an OMe group in the aromatic side-chain.

II. Experimental

(a) Synthesis of Ac–Leu–Val–Tyr(Me)–NHMe

This peptide was synthesized as an extension of the tripeptide model Ac–Val–Tyr(Me)–NHMe already presented before.[12] Similarly, *N-tert*-butyloxycarbonyl-*O*-methyl-(*S*)-tyrosine (295 mg, 1.00 mmol) was first converted into its *N*-methyl amide after activation with CDI and subsequent treatment with methylamine in THF (250 mg, 0.81 mmol, 81%). After Boc deprotection with dry TFA (5 mL) in dichloromethane and evaporation of the solvent, the free ammonium salt was obtained in quantitative yield. 322 mg (1.00 mmol) of this was coupled to *N*-Boc-(*S*)-valine using HATU/HOAt and lutidine in dichloromethane–DMF and produced, after aqueous workup, filtration and drying Boc–Val–Tyr(Me)NHMe (453 mg, 0.45 mmol, 53%). Boc deprotection was accomplished as before by stirring the starting material at 0 °C in a mixture of trifluoroacetic acid (5 mL) and dichloromethane (20 mL) for 4 h. After evaporation to 3 mL, the corresponding pure trifluoroacetate ammonium salt was precipitated by careful addition of cold dry diethylether (50 mL) in almost quantitative yield. This was finally coupled to *N*-acetyl-leucine (62 mg, 0.44 mmol) with HATU/HOAt and lutidine in dichloromethane–DMF and furnished, after the usual workup, 99 mg (0.22 mmol, 50%) of the desired tetrapeptide model *N*-acetyl-(*S*)-leucinyl-(*S*)-valinyl-(*S*)-O-methyltyrosinyl-*N*-methylamide [abbreviated as Ac–Leu–Val–Tyr(Me)NHMe] was obtained as a colorless solid.

(b) Experimental set-up

The experimental set-up has been described elsewhere (*cf.* ref. 6 and 23). 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 400), was used for excitation to the S_1 state and for ionization. The IR light in the region of 2.84–3.08 μm (3250–3520 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 (2.84–3.08 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. In order to obtain IR/R2PI spectra the IR laser is fired 60 ns prior to the UV laser. The substance (Ac–Leu–Val–Tyr(Me)–NHMe) and the valve are heated to 170 °C. Helium was used as carrier gas (2000 mbar).

III. Results and discussion

The R2PI spectrum of Ac–Leu–Val–Tyr(Me)–NHMe has been recorded in the range from 35 300–36 000 cm^{-1} yielding a broadened unstructured transition with two maxima at 35 578 cm^{-1} and 35 624 cm^{-1}. The IR/R2PI spectra recorded *via* the transitions at 35 578 and 35 624 cm^{-1} show well resolved IR transitions but the spectra are not identical. Thus two isomers of Ac–Leu–Val–Tyr(Me)–NHMe have to be discussed.

The IR spectrum of the first isomer (*cf.* Fig. 2) has been recorded *via* the electronic transition at 35 578 cm^{-1}. By taking into account that hydrogen-bonded NH groups are usually located below 3400 cm^{-1} and free NH groups have frequencies above 3400 cm^{-1}, the four observed IR frequencies of the first isomer can be divided in two groups: strongly bounded NH groups at 3357 and 3378 cm^{-1} and weakly bounded or free NH groups at 3418 and 3469 cm^{-1}. Thus the isomer must have at least two hydrogen bonds and one free NH group.

In comparison with this isomer the IR spectrum of the second isomer (*cf.* Fig. 3) looks completely different in the region below 3400 cm^{-1}. Only one very broad transition can be observed which is centred at 3319 cm^{-1}. The frequencies of 3417 and 3471 cm^{-1} are very similar to the frequencies of the first isomer at 3418 and 3469 cm^{-1}, but a third new band is observed at 3435 cm^{-1}.

In order to assign the isomers to distinct structures, calculations have been performed at various levels of theory with increasing accuracy. As a starting point to explore the potential energy surface a combined molecular mechanics/molecular dynamics approach has been applied using the class II force field CFF of Accelrys.[8,24] 2079 structures have been obtained at this level of theory. The following *ab initio* and DFT calculations have been performed using the Gaussian03 program package.[25] The 164 most stable structures from the force field analysis up to 24 kJ mol^{-1} above the minimum structure have been further optimised at the HF/3-21G* level. At this level of theory the highest relative energy is 51.1 kJ mol^{-1} (4276 cm^{-1}) with respect to the minimum structure. 27 of these structures, the five most stable ones, which are all combined γ/β-turns, and the most stable ones for other types of secondary structure binding motifs have been reoptimised and

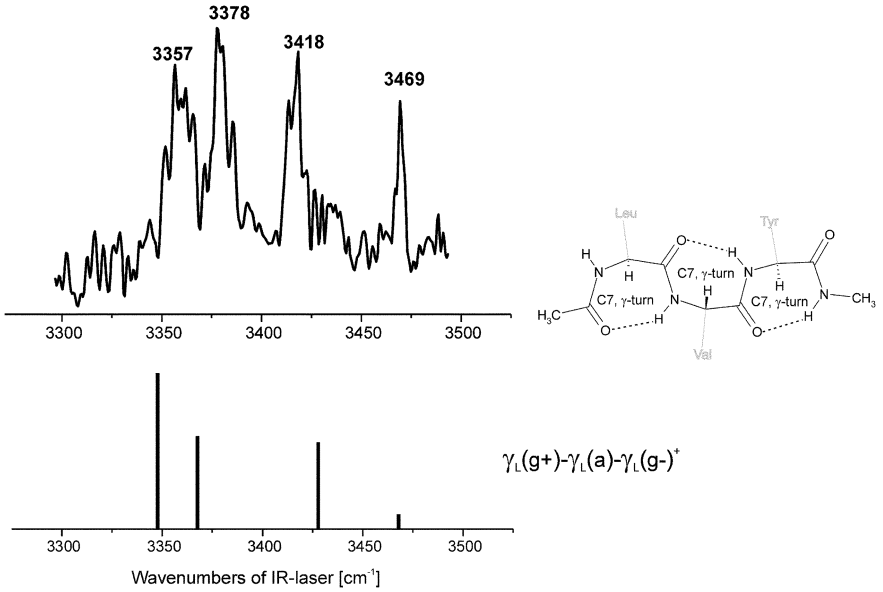

Fig. 2 IR/R2PI spectrum of isomer I in the range from 3300–3500 cm^{-1} recorded *via* the electronic transition at 35 578 cm^{-1}. The calculated harmonic frequencies of the assigned conformer are shown in the lower part and a schematic picture is depicted on the right side, too. The frequencies obtained from B3LYP/6-31 + G* calculations have been scaled with 0.9605.[12]

harmonic frequencies have been calculated at the B3LYP/6-31 + G* level. Due to the fact that the recorded IR spectra yield the information that for both isomers at least one NH group is involved in a hydrogen bond, the other selected isomers besides the five most stable ones represent different types of secondary structure binding motifs which contain at least one hydrogen bond. These types are: double β-turn (3_{10} helix), single γ-turn, double γ-turn, and triple γ-turn (2_7 ribbon) arrangements. For comparison extended β-sheet related structures have also been calculated. Surprisingly, no single β-turn has been found among the 966 most stable force field structures. Finally, single point calculations at the MP2/6-311 + G* level have been performed on the structures optimised at the B3LYP/6-31 + G* level. The geometries

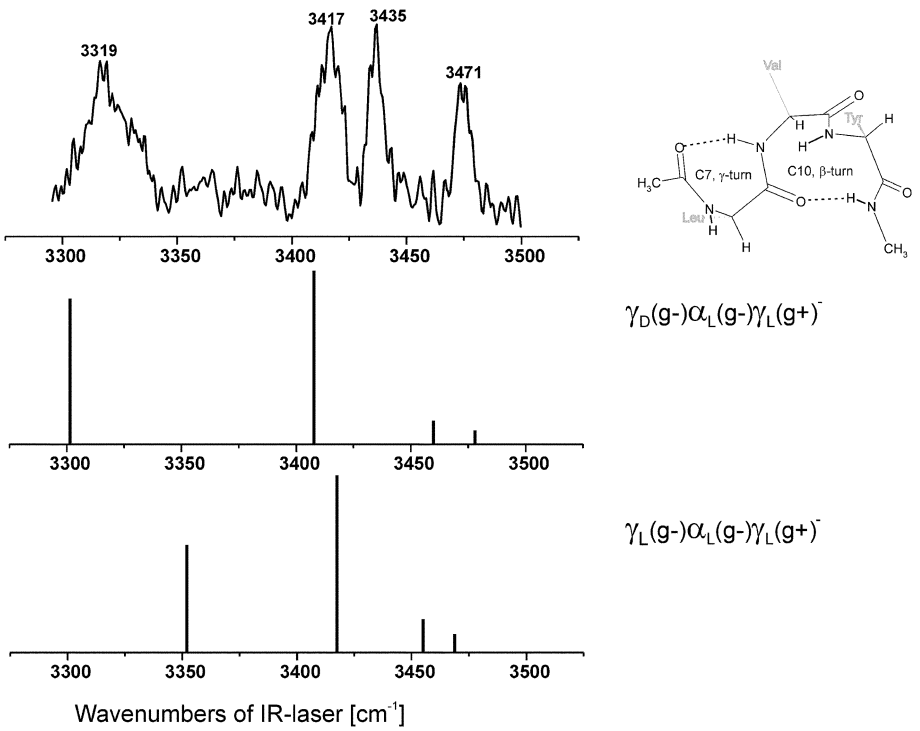

Fig. 3 IR/R2PI spectrum of the second isomer recorded *via* the electronic transition at 35 624 cm^{-1}. The scaled frequencies (B3LYP/6-31 + G*, f = 0.9605[12]) of the two γ–β conformers which fit best to the experimental data are also shown in the lower trace. A schematic picture of this structure type is shown on the right side.

according to the peptide nomenclature, relative energies and harmonic frequencies are listed in Table 1 for the most stable isomers of the aforementioned types of secondary structures. Some of the calculated structures are shown in Fig. 4. As reported in earlier papers the Greek letters refer to different backbone arrangements according to the Ramachandran plot[26,27] defined by the angles ϕ and ψ (*cf.* Fig. 1). The abbreviations a, g+, g− describe different positions of the side-chain defined by the angle χ_1. In the case of tyrosine (Tyr(Me)) the additional suffix ± (*cf.* Table 1) describes the position of the OMe group which can be rotated by 180° with respect to the C–O axis between the aromatic chromophore and the O of the OMe group.

Interestingly, the relative energies of all conformers listed in Table 1 except the combined γ–β-turns become much higher when going from the DFT to the MP2 level. Due to the lack of optimisation at the MP2 level, dispersion interaction which is not involved in DFT calculations can be overestimated. This phenomenon has also been observed recently by Compagnon *et al.*[28] in the investigation on Z–Aib–Pro–NHMe. In this work β-turns are the most stable structures, too. Nevertheless, from the IR data these structures could be excluded. In a very recent work van Mourik *et al.*[29] reported on the sensitivity of flexible molecules concerning their conformation and relative stability to the type of electron correlation used in calculations. They concluded that large basis sets are needed to circumvent intramolecular basis set superposition errors which can cause large energy shifts in MP2 calculations.

However, it can be concluded that with respect to the DFT calculations several structural arrangements of similar energy have to be discussed. β-Sheet related structures as well as combined γ-turn/β-turns and triple γ-turns structures may be formed. Since the two experimentally observed IR spectra show at least one IR transition that is located significantly below 3400 cm^{-1} a completely β-sheet related structure like in Ac–Val–Phe–OMe or Ac–Val–Tyr(Me)–NHMe can be excluded, *i.e.* in a β-sheet related structure only free NH stretching frequencies can be observed.

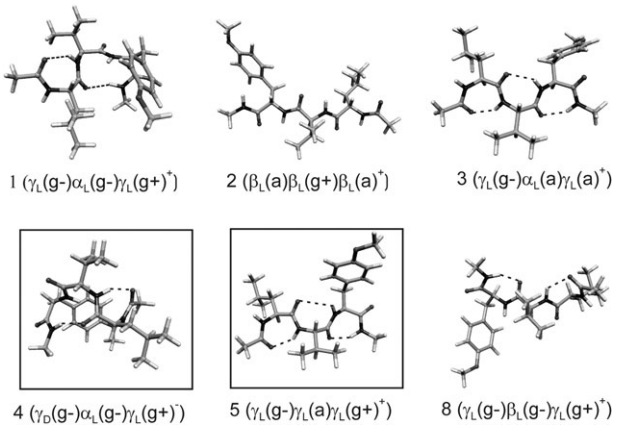

Fig. 4 Calculated structures of Ac–Leu–Val–Tyr(Me)–NHMe at the B3LYP/6-31+G* level of theory according to the nomenclature of Table 1. The most stable isomers are shown for the following secondary structure types: γ–β-turns, β-sheet related, triple γ-turn and double γ-turn. The two corresponding structures of the experimentally observed isomers I and II are marked with frames.

The IR/R2PI spectrum of isomer I shows two strong transitions at 3378 and 3357 cm^{-1} that can be related to two strongly hydrogen-bonded NH groups. This may lead to the conclusion that this is due to a conformer with a double-γ-turn. Nevertheless, the vibrational patterns predicted for the three different types of double γ-turns (β–γ–γ; γ–β–γ; γ–γ–β) are not in good agreement to the experimental spectrum. The best agreement for these types of structures is obtained for isomer 10 (β–γ–γ). The frequencies calculated for the NH_{Val}, NH_{Tyr}, and NHMe stretching modes fit very well to the experimental values but the frequency predicted for the NH_{Leu} group deviates by 24 cm^{-1} (*cf.* Table 1). The Leu residue is in a β_L conformation and it should be pointed out that for this type of structure in an Ac–X (X = amino acid) binding motif, the NH stretching vibration is usually located at about 3440 cm^{-1}, *e.g.* in Ac–Phe–NHMe,[8] Ac–Trp–NHMe,[5] Ac–Val–Phe–OMe[10] or Ac–Val–Tyr(Me)–NHMe[12] the corresponding NH stretching vibrations of Phe, Trp, and Val deviate only a few

Table 1 Relative energies (including zero point energies) and scaled harmonic frequencies (scaling factor: 0.9605 [12]) at the DFT level of theory using the B3LYP functional and the 6-31+G* basis set. Intensities are given in km mol^{-1}. Single point MP2 energies have been calculated at the DFT optimised structures using the 6-311+G* basis set. Frequencies of assigned isomers are given in bold letters

Geometries			Rel. energies/kJ mol^{-1}		Calculated vibrations [cm^{-1}] and intensities [km mol^{-1}]			
Isomer	Conformation	Secondary structure	DFT	SP-MP2	NH-Leu	NH-Val	NH-Tyr	NHMe
1	γ_L(g−)α_L(g−)γ_L(g+)$^+$	γ-turn-β-turn	0	0.92	3469(25)	3353(141)	3456(44)	3417(230)
2	β_L(a)β_L(g+)β_L(a)$^+$	β-sheet related	1.55	36.87	3438(62)	3426(86)	3416(98)	3468(50)
3	γ_L(g−)γ_L(a)γ_L(a)$^+$	Triple γ-turn	5.57	37.33	3468(23)	3362(151)	3382(110)	3393(114)
4	γ_D(g−)α_L(g−)γ_L(g+)$^-$	γ-turn-β-turn	6.16	0	**3478(23)**	**3302(227)**	**3460(38)**	**3408(270)**
5	γ_L(g−)γ_L(a)γ_L(g+)$^+$	Triple γ-turn	8.72	33.23	**3468(21)**	**3348(230)**	**3428(137)**	**3368(128)**
6	ε_D(g−)α_L(g−)γ_L(g−)$^+$	Double β-turn	11.08	10.44	3488(30)	3487(29)	3393(202)	3424(186)
7	γ_L(a)γ_L(g−)β_L(a)$^+$	Single γ-turn	12.53	41.65	3465(23)	3448(49)	3416(92)	3469(48)
8	γ_L(g−)β_L(g−)γ_L(g+)$^+$	Double γ-turn	12.86	40.08	3467(21)	3423(22)	3425(163)	3361(208)
9	γ_L(g−)γ_L(g−)β_L(a)$^-$	Double γ-turn	12.99	41.69	3468(22)	3378(120)	3357(125)	3475(25)
10	β_L(g−)γ_L(g−)γ_L(g−)$^+$	Double γ-turn	14.50	34.57	3442(52)	3470(35)	3351(158)	3383(146)
11	β_L(g+)β_L(g−)γ_L(g−)$^+$	Single γ-turn	14.62	40.73	3440(79)	3427(47)	3456(37)	3392(149)
12	β_L(g+)γ_L(g−)β_L(a)$^+$	Single γ-turn	22.50	40.04	3439(82)	3472(31)	3371(90)	3469(32)
			Experimental vibrations					
			Isomer I (assigned to isomer 5)		**3469**	**3357**	**3418**	**3378**
			Isomer II (assigned to isomer 4)		**3471**	**3319**	**3435**	**3417**

wavenumbers from 3440 cm^{-1}. The band position of 3442 cm^{-1} calculated for the NH_{Leu} stretching mode in the β_L conformation is therefore in good agreement with the expected value for this binding motif, but it is not in agreement with the experimental value of 3418 cm^{-1}.

Another prominent secondary structure binding motif, the double β-turn (3_{10} helix), can be excluded due to its vibrational pattern which is quite different from the experimental IR spectrum (*cf.* Table 1).

By comparing the calculated harmonic frequencies with the experimental values obtained for isomer I by far the best fit is obtained for isomer 5 (*cf.* Fig. 2). This isomer adopts a triple γ-turn ($\gamma_L(g-)\gamma_L(a)\gamma_L(g+)^+$) arrangement in which the middle residue, valine, is not so strongly folded like the two outer residues leucine and tyrosine. This is indicated by the long distance of 2.91 Å (calculated at the DFT level) between the carbonyl group of leucine and the NH group of tyrosine. Thus only a very weak interaction is possible, leading to a relatively high NH stretching frequency of 3418 cm^{-1} that is well predicted by the DFT calculations (3428 cm^{-1}). Furthermore the N–H···O angle of the intramolecular hydrogen-bond is only 110°. For comparison, the two NH groups of isomer 5 that are strongly hydrogen-bonded to the carbonyl groups of valine and the acetyl group have distances of 1.97 and 2.12 Å and N–H···O angles of 150° and 139°, respectively. This leads to strong red shifts of the NH stretching frequencies to 3378 and 3357 cm^{-1} which are also well predicted by the DFT calculations (3368 and 3348 cm^{-1}). Saturation effects due to large IR power cannot be completely excluded and may have an influence on the shape of the bands, nevertheless the calculated intensities reproduce the order and magnitude of the experimental bands in which free and hydrogen bonded species can clearly be distinguished by their different bandwidths.

All hydrogen bond distances and angles are listed in Table 2. To our knowledge Ac–Leu–Val–Tyr(Me)–NHMe is the first species investigated up to now that forms a triple γ-turn structure. This secondary structure element contains the so-called 2_7 ribbon motif, which is a special helical type consisting of consecutive seven membered rings.

While a triple γ-turn arrangement is predicted for isomer I, the experimentally observed frequencies of isomer II (*cf.* Fig. 3) fit best to a combined γ–β-turn structure. Two isomers (1 and 4) of this type belong to the most stable structures (*cf.* Table 1). Both isomers contain a β-turn type I arrangement[30] similar to the one assigned for Ac–Ala–Ala–Phe–NH_2.[20] This tripeptide exhibits similar frequencies as the ones observed for isomer II of Ac–Leu–Val–Tyr(Me)–NHMe. The calculated structures of isomers 1 and 4 of Ac–Leu–Val–Tyr(Me)–NHMe differ only by the orientation of the leucine residue which is γ_D in isomer 4 and γ_L in isomer 1, representing the reverse and classical γ-turn. (Note: The abbreviations γ_D and γ_L refer to different arrangements with respect to the Ramachandran plot,[26] D and L should not be mixed up with the absolute configuration of the C_α atom which is always *S*.) For the transition of lowest frequency centered at 3319 cm^{-1}, the calculated NH stretching frequency of the valine moiety fits better to isomer 4 (3302 cm^{-1}) than to isomer 1 (3353 cm^{-1}). The three other NH stretching frequencies calculated for both isomers have similar values which deviate by not more than 9 cm^{-1} from each other. The transition with the lowest frequency at 3417 cm^{-1} is assigned to the NHMe protection group which is involved in the hydrogen bond formed within the β-turn. The transitions at 3435 and 3471 cm^{-1} can only be assigned to the free NH groups of the tyrosine and leucine residues. The calculated NH stretching vibration of the tyrosine residue shows the strongest deviation from the experimental values. In both isomers 1 and 4 the calculated frequency is too high. This deviation which has also been observed for other calculated β-turn structures using the B3LYP/6-31+G* method[20] might be an underestimation of the π-interaction with the carbonyl group of the amide bond. Other conformers with a single γ-turn are either energetically much less favoured or show no good agreement with the experimental frequencies, *e.g.* the red shift of the bounded NH group is much too small in all cases

Table 2 Distances and angles calculated at B3LYP/6-31+G* level of theory for two different types of hydrogen bonding: C10 for β-turns and C7 for γ-turns. The isomers represent the structures: γ-turn/β-turn (isomers 1 and 4), triple γ-turns (3, 5) and double γ-turns (8, 9, 10). The indices of the atoms correspond to the nomenclature depicted in Fig. 1. Additionally the calculated NH stretching frequencies are given for every isomer, according to Table 1

	Type of hydrogen bonding									
	C10 (β-turn)					C7 (γ-turn)				
Isomer	*r*/Å		∠/°		Freq./cm^{-1}	*r*/Å		∠/°		Freq./cm^{-1}
1	$N_4H_4\cdots O_2{=}C_2$	2.13	$N_4{-}H_4{-}O_2$	159.7	3417	$N_2H_2\cdots O_1{=}C_1$	2.08	$N_2{-}H_2{-}O_1$	143.6	3353
3						$N_2H_2\cdots O_1{=}C_1$	2.1	$N_2{-}H_2{-}O_1$	140.9	3362
						$N_3H_3\cdots O_2{=}C_2$	2.17	$N_4{-}H_4{-}O_2$	136.4	3382
						$N_4H_4\cdots O_3{=}C_3$	2.14	$N_4{-}H_4{-}O_3$	140.2	3393
4	$N_4H_4\cdots O_2{=}C_2$	2.08	$N_4{-}H_4{-}O_2$	158.5	3408	$N_2H_2\cdots O_1{=}C_1$	1.9	$N_2{-}H_2{-}O_1$	152.6	3302
5						$N_2H_2\cdots O_1{=}C_1$	2.12	$N_2{-}H_2{-}O_1$	139.4	3348
						$N_3H_3\cdots O_2{=}C_2$	2.91	$N_3{-}H_3{-}O_2$	110.9	3428
						$N_4H_4\cdots O_3{=}C_3$	1.97	$N_4{-}H_4{-}O_3$	150	3368
8						$N_2H_2\cdots O_1{=}C_1$	2.37	$N_2{-}H_2{-}O_1$	128.1	3423
						$N_4H_4\cdots O_3{=}C_3$	2	$N_4{-}H_4{-}O_3$	148.3	3361
9						$N_2H_2\cdots O_1{=}C_1$	2.12	$N_2{-}H_2{-}O_1$	141.5	3378
						$N_3H_3\cdots O_2{=}C_2$	2.14	$N_3{-}H_3{-}O_2$	141.1	3357
10						$N_3H_3\cdots O_2{=}C_2$	2.06	$N_3{-}H_3{-}O_2$	144	3351
						$N_4H_4\cdots O_3{=}C_3$	2.04	$N_4{-}H_4{-}O_3$	142.9	3383

(*cf.* Table 1). Thus only a γ–β-turn arrangement, most probably a $\gamma_D(g-)\alpha_L(g-)\gamma_L(g+)^-$ (isomer 4), can be predicted for the second isomer of Ac–Leu–Val–Tyr(Me)–NHMe. It should also be mentioned that the γ–β-turn arrangement is the most stable structure both at the DFT level as well as on all calculated levels of *ab initio* theory (HF, MP2). For the sake of comparison the stick spectra of both isomers 1 and 4, which are possible candidates for isomer II, are depicted in Fig. 3.

IV. Conclusion

The tetrapeptide model Ac–Leu–Val–Tyr(Me)–NHMe has been investigated in the gas phase by applying IR/R2PI spectroscopy. Two isomers have been observed and the structures have been assigned by comparison with theoretical results. None of the isomers has a stretched backbone and it turns out that the extension of the smaller Ac–Val–Tyr(Me)–NHMe by the amino acid leucine leads to a strong preference of folded isomers. One isomer contains two hydrogen bonds by forming a combined γ-turn/β-turn arrangement. In the case of the second isomer a triple γ-turn arrangement (2_7 ribbon) can be observed for the first time. Both isomers have in common that the leucine residue adopts a γ-turn. Further investigations will show if the dimer of Ac–Leu–Val–Tyr(Me)–NHMe forms a β-sheet arrangement similar to the dimer of Ac–Val–Tyr(Me)–NHMe.[31]

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