Supporting Material: Intramolecular Amide Stacking and its Competition with H-bonding in a Small Foldamer

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2. METHODS

Synthesis

Ac-γ^2-hPhe-NHMe was prepared from the corresponding β-substituted-δ-nitrobutanol derivative by a previously demonstrated Michael addition/reduction sequence.[1] Jones oxidation provided the corresponding γ-nitro butyric acid derivative. This γ-nitro butyric acid derivative (1 equiv) was added directly to a solution containing methyl amine (1.2 equiv), (*N,N*-dimethylamino)propyl-3-ethylcarbodiimide hydrochloride (1.2 equiv), and *N,N*-diisopropylethylamine (1.2 equiv) in CH_2Cl_2. The resulting solution was stirred for 2 days at room temperature. The reaction mixture was diluted with EtOAc, and the resulting solution was washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and then brine. The organic layer was dried over $MgSO_4$, filtered and concentrated to give the desired amide, which was purified via SiO_2 column chromatography. The nitro group was subsequently reduced via hydrogenation with 10% palladium on activated carbon in methanol at an H_2 pressure of 50 psi to yield the corresponding amine, which was not purified. The amine was dissolved in CH_2Cl_2, and *N, N*-diisopropylethylamine (1.2 equiv) and acetic anhydride (1.2 equiv) were added at 0 °C. The mixture was stirred at room temperature for 3 hours and concentrated to give a crude product, which was purified via column chromatography eluting with CH_2Cl_2/MeOH. The product was further purified by recrystallization from a methanol/EtOAc/hexane mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28-7.11 (m, 5H), 6.52 (q, J = 4.9 Hz, 1H), 6.24 (t, J = 4.9 Hz, 1H), 3.37 (m, 1H), 3.10 (m, 1H), 2.96, 2.66 (AB of ABX, J_{AB} = 13.4 Hz, J_{AX} = 8.4 Hz, J_{BX} = 5.9 Hz, 2H) , 2.68 (d, J = 4.9 Hz, 3H), 2.44 (m, 1H), 1.94 (s, 3H), 1.86 (m, 1H), 1.59 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.57, 171.18, 139.78, 129.06, 128.60, 126.53, 47.00, 39.26, 38.05, 32.73, 26.29, 23.40; HRMS *m/z* (ESI): calc. for $C_{14}H_{20}N_2O_2Na$ $[M+Na]^+$ 271.1417, found 271.1429.

Experimental Methods

The solid peptide sample was wrapped in glass wool and placed in a glass insert in an effort to reduce thermal decomposition, then inserted into a stainless steel sample holder and heated to approximately 175 °C to increase the sample vapor pressure. The sample holder was located directly behind a pulsed valve (Parker General Valve, Series 9), fitted with a 400 μm nozzle orifice. For the basic spectroscopic measurements the sample was entrained in a carrier gas of 70/30 % neon/helium mixture with a backing pressure of 1.5 bar and expanded into vacuum. The sample molecules were collisionally cooled through collisions with the carrier gas and skimmed, via a conical skimmer (Beam Dynamics Inc., 55° conical angle, 2 mm diameter), prior to entering the ionization region of a time-of-flight-mass-spectrometer (TOF). Low total gas flow rates (2-4 bar·cm^3/min) were utilized to minimize interference of the gas pulse with the conical skimmer.

Resonant two-photon ionization (R2PI) spectroscopy was used to record the electronic spectra of the isolated molecules. The molecules were excited with the frequency doubled output of a Nd:YAG pumped tunable dye laser. Typical ultraviolet laser powers used were 0.1 – 0.5 mJ/pulse. The ultraviolet light traversed the ionization region of the TOF as a collimated beam of approximately 1 mm diameter. The resultant ions were detected by a micro-channel plate detector (Jordan TOF, 2.5 cm) fixed atop a one meter long flight tube.

Conformation-specific infrared spectra were recorded using the double resonance method of resonant ion-dip infrared (RIDIR) spectroscopy. Tunable infrared radiation was obtained using a seeded, Nd:YAG pumped parametric converter (LaserVision, KTA based). Infrared laser powers were 3-5 mJ/pulse. In order to extend the tuning range of the infrared laser system to the Amide I spectral region (1600-1800 cm^{-1}), difference-frequency mixing of signal and idler from the KTA stage of the parametric converter using a $AgGaSe_2$ crystal. Typical output powers of 150-200 μJ/pulse of mid-infrared were generated from a combined 15-20 mJ/pulse of signal and idler.

The IR (10 Hz) and UV probe (20 Hz) laser beams were counter-propagated, spatially overlapped, and temporally separated by 200 ns, with the IR preceding the UV probe pulse. With the UV laser wavelength fixed on a particular transition due to a single conformer, the difference in ion signal from the UV probe was monitored while the IR laser was tuned through the spectral region of interest. When the IR laser was resonant with a vibrational transition arising from the same ground state as that being probed by the UV laser, a depletion in the ion signal was observed. The IR spectrum was recorded by a personal computer recording the output of a gated integrator (Stanford Research Systems) in active baseline subtraction mode.

Mass-resolved infrared population transfer spectra were obtained by counterpropagating the molecular beam and an infrared excitation pulse, thereby creating a column of IR-excited molecules for interrogation. By adjusting the timing of the IR laser excitation relative to the UV probe laser (Δt=44 μsec), molecules initially excited within a few mm of the pulsed valve orifice can isomerize and be re-cooled as they travel to the ion source region for interrogation with the UV probe via R2PI. The ion signal due to one conformer is monitored via its S_0-S_1 origin transition while the IR laser is tuned through the spectral region of interest, the amide NH stretch region (3300-3500 cm^{-1}). IR transitions due to the probed conformer appear as depletions in ion signal, while gains in

ion signal are observed when transitions of other conformers are encountered that transfer population into the probed conformer zero-point level.

The MR-IRPT spectra display a certain reflection symmetry in that gains in one spectrum are compensated for by depletions in another. If re-cooling is complete, then averaging over all infrared wavelengths should indicate that there is no net change in the total population of Ac-γ^2-hPhe-NHMe, but rather a redistribution of the population amongst the three conformers. The set of MR-IRPT spectra can be used to extract relative abundances by taking weighted sums of the MR-IRPT spectra that zero-out the net change in population at all infrared wavelengths ($\Delta N_{tot}(\tilde{\nu}) = 0$). Since the population transfer spectra are reported as fractional population changes in each conformer *X* ($I_{PT}^{X}(\tilde{\nu}) = \frac{\Delta N_X(\tilde{\nu})}{N_X}$), we have

$$\Delta N_{tot}(\tilde{\nu}) = 0 = \sum_{X=A,B,C} F_X \cdot I_{PT}^{X}(\tilde{\nu}),$$

where F_X is the fractional abundance of conformer X (i.e., $F_X = N_X/N_{tot}$). Thus, the weighted sum that is zero at all infrared wavelengths has weighting factors equal to the fractional abundances of the conformers F_X.

Computational Methods

For a molecule the size of Ac-γ^2-hPhe-NHMe, it is important to do a systematic search of conformational space initially in order to find all possible structural motifs, with an initial assessment of their relative energies. Such a search was first carried out using the MMFFs force field[2] within the MACROMODEL[3] suite of programs. The 114 conformational minima so found within a 50 kJ/mol window were then used as starting structures for higher level computations to compute optimized geometries, relative energies, vibrational frequencies, and infrared intensities. Initial calculations employ density functional theory with the B3LYP functional[4,5] and a 6-31+G(d) basis set, using the GAUSSIAN 03 suite of programs.[6] The vibrational frequencies are then compared to the experimentally recorded, conformation-specific infrared spectra and tentative structural assignments are made.

Since it has been shown that the B3LYP functional does not accurately account for dispersive interactions, the DFT B3LYP calculations were followed up with calculations at the DFT M05-2X level, which has been developed by Truhlar and co-workers explicitly to better account for dispersive interactions, using the scf = tight and ultrafine grid options.[7] This level of theory has been shown in several recent studies to provide a more accurate set of relative energies for the minima. The resultant DFT M05-2X vibrational frequencies were then compared to the experimental infrared spectra leading to the firm assignments of the resolved conformers. It should be noted that the structural assignments remain the same as the tentative assignments

Single-point TDDFT vertical excitations were computed at the M05-2X/6-31+G(d) level of theory. The resultant frequencies for the lowest singlet excited state were scaled by 0.834, chosen to match the calculated excitation energy of the C9(g_-) structure with that recorded experimentally for conformer B.

Systematic Fragmentation Method/Effective Fragment Potential

The systematic fragmentation method (SFM) relies on the assumption that chemistry is largely a local phenomenon. This justifies the notion that a molecule (M) can be described simply as a collection of functional groups (eq 1). SFM is able to fragment this general chemical system M into chemically relevant functional groups G_i.

$$M = G_1G_2G_3...G_k \quad (1)$$

SFM subsequently combines the properties of the individual (hydrogen capped) functional groups to recover the properties (e.g., energies) of the full (un-fragmented) system. There are two energy terms accounted for through SFM, a bonded (E_{bonded}) and a non-bonded $(E_{non\text{-}bonded})$ term. The bonded term accounts for the sum of the energies of the fragments. The non-bonded component accounts for the interactions between the fragments, in their original positions prior to fragmentation.

To recover the interaction energy between non-bonded functional groups, a many-body expansion is employed. This expansion is normally terminated at the two- or three-body terms, with the higher order terms corresponding to higher accuracy (e.g., the three-body expansion includes two-body interactions). In the present study, only two-body terms are required, since the amide groups contained in the systems of interest are individual functional groups in the SFM treatment. The two-body interaction is determined by a super-molecular calculation that includes the two functional groups with subsequent subtraction of the isolated group energies (eq 2), to avoid double counting.

$$E_{nb}^{(1,1)}[G_1;G_2] = E(G_1G_2) - E(G_1) - E(G_2) \quad (2)$$

For a large molecular system, the number of non-bonded interactions can become very large[8], so evaluating these interactions with the same level of quantum chemistry as is used for the bonded terms can be computationally prohibitive. A promising alternative is to evaluate the non-bonded interactions using the effective fragment potential (EFP) method. It has been demonstrated that the EFP method can very accurately reproduce correlated electronic structure theory predictions for non-bonded interactions, as long as the interacting fragments are not so close that one is high on the inter-fragment potential wall.[9] The EFP method is a semi-classical model that is very computationally efficient and does not suffer from basis set superposition error (BSSE). The EFP interaction energies have contributions from electrostatics (Coulomb), polarization (induction), exchange-repulsion, dispersion and charge transfer (eq 3). This feature of EFP is of great importance for the analysis of the interactions between the interacting amide groups.

$$E_{interaction}^{EFP} = E_{electrostatics} + E_{polarization} + E_{exchange-repulsion} + E_{dispersion} + E_{charge-transfer} \quad (3)$$

The combined use of SFM and EFP reveals the dominant interactions between all of the groups for the molecules considered in this work. The interactions of interest in this work are those between the neighboring amide groups; the results are summarized in

Table 1. For structure C, the electrostatic + exchange repulsion interaction energy (a repulsive +4.5 kJ/mol) is more than offset by the attractive dispersion interaction. So, it can be concluded that dispersion contributes significantly to the stability of structure C. The dispersion interaction is not quite so large for structures A and B, with electrostatics exhibiting a significant contribution toward the stabilization energy. It is evident that the stability of structures A and B results from a combination of electrostatics, dispersion and polarization.

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3. Further details of the conformational assignments

The evidence for amide stacking over extended conformers has three components to it.

(1) The single-conformation IR spectra in the amide NH stretch and amide I regions are fit remarkably well by the amide stacked structure. In particular, the spacing and relative intensities of the amide NH stretch fundamentals match well (**Figure 1**), while the amide stacked structure has only a single observed transition, in keeping with the calculated six-fold reduction in the intensity of the lower-frequency C=O stretch fundamental (**Table S1**).

By comparison, **Figure S1** contains the calculated results for the three lowest-energy extended structures (all labeled Ex(a) since all three have the phenyl ring in the *anti* position). These extended structures have the wrong spacing between the amide NH stretch fundamentals. The amide I region is only plausibly fit by one of the extended structures (Ex(a), assuming that the two C=O stretch fundamentals are unresolved in the experimental spectrum), but previous experience indicates that even shifts of 1-2 cm^{-1} show up as a shoulder or doublet under expansion-cooled experimental conditions such as used here.

(2) The lowest energy extended structures are all at least 10 kJ/mol higher in energy than the amide stacked structures, and assigning the spectrum to an extended structure would then beg the question as to why we do not observe the amide stacked structure.

(3) TDDFT calculations of the relative S_0-S_1 transition energies show an excellent match between the transition energies of conformers C9(*a*), C9(g_), and S(a) and the frequency separation for conformers A, B, and C. Note that in making this comparison, we have scaled the TDDFT calculations so that the experimental and calculated energy separations match for conformer B. After doing so, the relative positions of conformers A (+111 cm^{-1}) and C (-12 cm^{-1}) are within 11 and 1 cm^{-1} of the experimental values. This is visualized in **Figure 1** in the main text, while the quantitative values are included below in **Table S1**. In contrast, the fully extended structures are calculated to be shifted more than 300 cm^{-1} to the red (**Table S1**), well outside the experimental range investigated.

Table S1: Comparison of the experimental UV and IR spectral data (S_0-S_1 origin, amide I (C=O), amide NH stretch frequencies, R2PI relative abundances and MRIRPT relative abundances) with calculated results (single-point TDDFT M05-2X/6-31+G(d) scaled vertical excitation energies, scaled, harmonic amide I (C=O) and amide NH stretch vibrational frequencies and infrared intensities.

	Family	Assignment	S_0-S_1 cm^{-1}	R2PI Intensity	Expt. C=O cm^{-1}	Calc. C=O[a] cm^{-1} (Intensity km/mol)	Expt. NH cm^{-1}	Calc. NH[b] cm^{-1}	Fract. Abundance MRIRPT	Rel. Energy kJ/mol	TDDFT (SP)[c] cm^{-1}
Ac-γ^2-hPhe-NHMe											
A	C9	C9(a)	37584 (+100)	0.55	1692 1709	1692 (283) 1710 (284)	3372 3481	3391 3478	0.38 ± 0.01	0.97	37595 (+111)
B	C9	C9(g-)	37484 (0)	0.40	1693 1710	1690 (337) 1710 (286)	3357 3476	3371 3472	0.41 ± 0.02	0.00	37484 (0)
C	S	S(a)	37471 (-13)	0.05	1716	1710 (60) 1716 (369)	3469 3480	3462 3478	0.21 ± 0.01	1.31	37472 (-12)
	C7	C7(a)	-	-	-	1694 (312) 1709 (313)	-	3345 3462	-	11.25	37457 (-27)
	Ex	Ex(a)	-	-	-	1710 (306) 1716 (249)	-	3454 3482	-	10.26	37066 (-418)
	Ex	Ex(a)	-	-	-	1718 (220) 1721 (276)	-	3449 3483	-	12.46	36542 (-942)
	Ex	Ex(a)	-	-	-	1712 (179) 1718 (311)	-	3463 3490	-	13.75	37182 (-302)

[a]Computed DFT M05-2X/6-31+G(d) C=O stretch (amide I) frequencies scaled by 0.96

[b]Computed DFT M05-2X/6-31+G(d) amide NH stretch frequencies scaled by 0.94

[c]Computed TDDFT(SP) M05-2X/6-31+G(d) S_0-S_1 vertical separations, scaled by 0.834 in order to bring the calcualted separation for C9(g-) to match up with the experimental S0-S1 origin of conformer B

Figure S1. Left: RIDIR spectrum for conformer C of Ac-γ^2-hPhe-NHMe (top trace) and stick diagram of the scaled (0.96), harmonic vibrational frequencies and infrared intensities in the amide I spectral region of the amide stacked structure (red sticks) and three low energy fully extended conformers (black sticks) at the DFT M05-2X/6-31+G(d) level of theory. Right: RIDIR spectrum for conformer C of Ac-γ^2-hPhe-NHMe (top trace) and stick diagram of the scaled (0.96), harmonic vibrational frequencies and infrared intensities in the amide NH spectral region of the amide stacked structure (red sticks) and three low energy fully extended conformers (black sticks) at the DFT M05-2X/6-31+G(d) level of theory.