A Time-resolved Spectroscopic Comparison of the Photoisomerization of Small β-Turn-forming Thioxopeptides

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The monosubstituted thioxopeptide Boc-Ala-Pro-ψ(SC—NH)-Aib-Ala-OMe is investigated by time-resolved UV-pump/IR-probe and IR-pump/IR-probe spectroscopy, steady-state FTIR-spectroscopy, and NMR-techniques. The compound has a high propensity to adopt a $i \rightarrow i + 3$ hydrogen-bonded conformation. Time-resolved infrared measurements reveal the opening of this β-turn structure upon trans → cis photo isomerization of the thioamide bond. Comparison is made with three protected tripeptides containing the -SC—NH-Aib- moiety with different thio-substituted residues. Very similar photo isomerization dynamics and comparable quantum efficiencies are found. Differences are seen for the thermally activated cis → trans relaxation in the electronic ground state, where thioxopeptides with larger residues next to the thioamide moiety exhibit subsecond isomerization times. Anisotropy measurements indicate a very rigid Aib-containing core structure for all four thioxopeptides in acetonitrile solution.

I. Introduction

To trigger conformational changes in peptides, azobenzene-based photo switches are widely used.[1−8] A less invasive approach is the substitution of the backbone oxygen atom in one peptide unit with a sulfur atom. The $\pi \rightarrow \pi^*$ transition of the resulting thiopeptide unit is thereby red-shifted to about 260 nm (the same transition of an oxopeptide unit is found below 200 nm).[9] The thiopeptide unit, which is predominantly in the trans conformation in thermal equilibrium, can thus be selectively excited by an UV pulse, and trans → cis isomerization of the thiopeptide bond can take place at a well defined position in the peptide.[10,11] Although a single trans thiopeptide unit does not significantly alter the secondary structure (α-helix or β-sheet) of the parent oxopeptide,[12,13] it has recently been shown that trans → cis isomerization of a single thioamide bond can be sufficient to switch off enzymatic activity.[14] After photoisomerization, the metastable cis conformation of the thioamide bond in the electronic ground-state relaxes back to trans with time constants that depend on the amino acid sequence and range from seconds to many minutes.[11]

N-Methylthioacetamide (NMTAA), the simplest isomerizing thioamide, has been used to investigate the isomerization mechanism upon $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ excitation.[9,15−18] A quantum yield for trans → cis photoisomerization of 30−40% was found with an isomerization time of a few 100 ps. This is the lifetime of the lowest-lying electronically excited state, in which the molecules get trapped after photoexcitation and where, according to high-level ab initio calculations,[17] there is no significant energy barrier but also no driving force to isomerization. Time-resolved UV/vis measurements indicate isomerization of the thioamide bond in larger monosubstituted thioxopeptides with up to 20 amino acids on the same time scale.[19] The efficient isomerization of the thioamide bond in larger peptides and in the presence of secondary structure is somewhat surprising in light of the ab initio results, which suggest a mechanism governed by diffusive motion about the NH—CS single bond in the electronic excited state.[17] To further investigate the mechanism and efficiency of isomerization in the presence of steric and conformational constraints, small monosubstituted thioxopeptides with a defined initial structure and a resolved vibrational spectrum in the amide I region are ideal model systems. In particular, β-turn conformations, which are the smallest secondary structure motifs and are ubiquitous in proteins, are ideally suited for this purpose. The formation of β-turns (and 3_{10}-helices) with a characteristic $i \rightarrow i + 3$ hydrogen bond in small peptides is favored by incorporation of the nonchiral residue Aib.[20,21] However, in our first study of this class of thioxopeptides we encountered a $i \rightarrow i + 4$ hydrogen-bonded loop structure in coexistence with more extended conformations for a glycine-containing tetrathioxopeptide.[22,23] To limit conformational heterogeneity and to stabilize the β-turn structure we here introduce a -Pro-ψ(SC—NH)-Aib- motif. The so-called "azirine/oxazolone-method" [20,21] was used to create the thiopeptide bond at the N-terminal side of the Aib residue. IR, NMR, and time-resolved IR spectroscopy confirms a dominant β-turn conformation for Boc-Ala-Pro-ψ(SC—NH)-Aib-Ala-OMe (**1a**), which opens upon isomerization of the thioamide bond.

Since Aib is relatively bulky and may perturb the isomerization process of the thiopeptide bond, we also investigated three peptides with different thio-substituted residues next to Aib: Z-Gly-ψ(SC—NH)-Aib-Ile-OMe (**2a**), Z-Ala-ψ(SC—NH)-Aib-Ile-OMe (**2b**), and Z-Ile-ψ(SC—NH)-Aib-Gly-OMe (**2c**). **2b** only differs in position $i + 1$ from **2a** (Ala instead of Gly), and in **2c** the residues $i + 1$ and $i + 3$ (Ile and Gly) are exchanged compared to **2a**. This series is used to study the effect of steric interactions on the degree of intramolecular hydrogen bonding and photoisomerization efficiency. For that purpose, time-resolved IR-spectroscopy is ideally suited, because it is not affected by the thermally activated cis → trans back-reaction in the electronic ground state. In addition, time constants of

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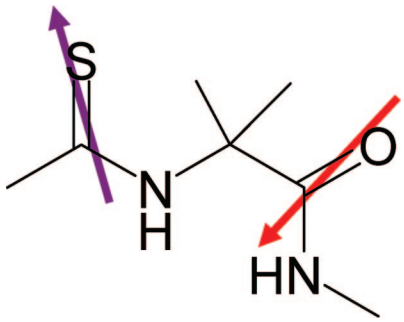

Figure 1. Schematic representation of the -ψ(SC—NH)-Aib- moiety common to all four thioxopeptides. Arrows indicate the orientation of the electronic $\pi-\pi^*$ and vibrational amide I transition dipole moments.

the isomerization dynamics can be measured, and structural information in solution can be obtained from polarization-dependent UV-pump/IR-probe spectroscopy. In the case of thioxopeptide **2c** this can be compared to a crystal structure.[20]

II. Experimental Methods and Materials

The thioxopeptides Z-Gly-ψ(SC—NH)-Aib-Ile-OMe (**2a**) and Z-Ala-ψ(SC—NH)-Aib-Ile-OMe (**2b**) were synthesized by Breitenmoser et al. (see ref 20), and Z-Ile-ψ(SC—NH)-Aib-Gly-OMe (**2c**) was synthesized by Lehmann et al. (see ref 21) using the azirine/oxazolone method. The thioxopeptides Boc-Ala-Pro-ψ(SC—NH)-Aib-Ala-OMe (**1a**) and the isotope- labeled analogue Boc-Ala-ψ(O ^{13}CN)-Pro-ψ(SC—NH)-Aib-Ala-OMe (**1b**) were synthesized according to the same procedure as used for **2c**[21] but using other amino acids (see Supporting Information for details). For the time-resolved UV-pump/IR-probe measurements, the samples ($\approx$ 10 mM solutions of the thioxopeptides in deuterated acetonitrile) were circulated through a flow cell made of CaF_2 windows spaced 50 μm apart, at a rate sufficient to ensure complete exchange of sample volume between excitation pulses.

Femtosecond pulses for time-resolved measurements (1 kHz, 700 μm, 80 fs) were obtained from an amplified titanium/sapphire laser system (Spectra Physics), operating at 800 nm. For UV-pump/IR-probe measurements, UV-pulses for the excitation of the thioxopeptides at 267 nm were generated by frequency tripling. The third harmonic beam was then stretched to 700 fs duration by guiding it through 10 cm of fused silica in order to suppress undesired nonlinear effects in and photodamage of the cell windows.

Midinfrared pulses in the 1600−1800 cm^{-1} range (100 fs, 1 μJ, 200 cm^{-1} bandwidth) were produced in a home-built double-stage optical parametric amplifier (OPA) followed by frequency mixing in a $AgGaS_2$ (silver thiogallate) crystal.[24] The IR pulses were split into three parts. The main portion was used as the pump pulse for the IR-pump/IR-probe measurements. It was passed through a computer-controlled Fabry−Perot filter. In this way, narrow-band pulses were obtained, the center frequency of which could be varied (10 cm^{-1} fwhm, $\approx$ 100 nJ/pulse). The second part (the probe pulse, 50 nJ) was focused in spacial overlap with either the UV- or the IR-pump pulse in the sample cell. The third IR beam was used as a reference to correct for intensity fluctuations and crossed the flow cell approximately 500 μm further upstream. Probe and reference beams were dispersed in a spectrometer and detected with a double MCT array (2 $\times$ 32 pixels) on a single-shot basis with 4 cm^{-1} resolution. After averaging over 300 laser shots at a given pump−probe delay, the polarization of the pump pulses was switched from parallel to perpendicular with respect to the probe pulses, in order to obtain magic angle and anisotropy data.

For irradiation during FTIR measurements a KrF-excimer laser (248 nm) was used, which was directed onto the sample inside the FTIR-spectrometer.

^{1}H- and ^{13}C NMR spectra were recorded on a Bruker 500 MHz spectrometer in deuterated acetonitrile.

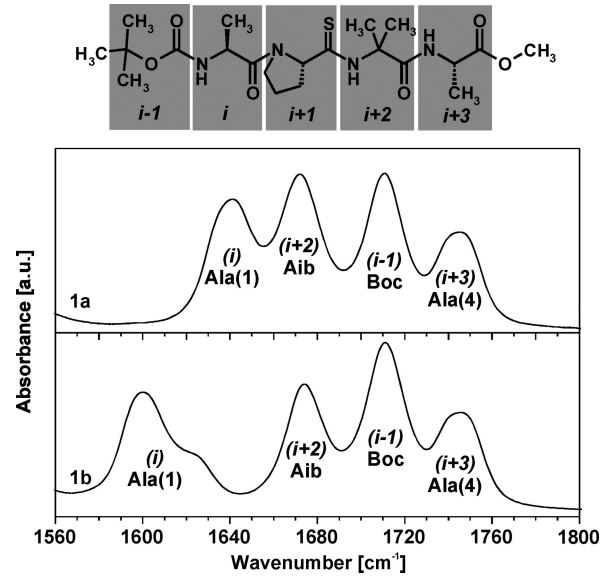

Figure 2. FTIR absorption spectra of the thioxopeptides **1a** and **1b** in CD_3CN at 293 K.

III. Results

A. Equilibrium Conformations of 1a and 1b. ***1. Infrared Absorption.*** The FTIR absorption spectrum of thioxopeptide **1a** in Figure 2 shows four bands in the 1700 cm^{-1} region, which can be assigned to the four C═O stretch modes of the molecule: amino acid i at 1641 cm^{-1} (Ala(1)), amino acid $i + 2$ at 1672 cm^{-1} (Aib), urethane protection group $i - 1$ at 1711 cm^{-1} (Boc) and amino acid $i + 3$ at 1745 cm^{-1} (Ala(4)). In the spectrum of the isotope labeled thioxopeptide **1b** the ^{13}C═O stretch band Ala(1) is red-shifted to 1600 cm^{-1} and a fifth, three times weaker band is visible at 1625 cm^{-1}, which is hidden in the spectrum of **1a**. We argue that these two bands are due to Ala(1) in $i \rightarrow i + 3$ hydrogen-bonded (lower frequency) and non-hydrogen bonded (higher frequency) conformations of the thioxopeptide. This assignment is consistent with temperature-dependent ^{1}H NMR and 2D-IR data.

2. ^{1}H NMR spectra. Figure 3a shows the ^{1}H NMR spectra of thioxopeptide **1a** in the 7 ppm region for temperatures between 253 and 313 K. The signal at 8.5 ppm is due the NH-proton of Aib (next to C═S), the peak at 7.0 ppm is due to Ala(4), and the one at 5.5 ppm is the amide proton signal of Ala(1). The amide protons of Aib and Ala(1) give rise to multiple peaks, which do, however, not reflect the splitting of the Ala(1) band in the FTIR spectrum. The integral intensity ratio of the two peaks of Aib is 9:1, which is reproduced in the ^{13}C NMR spectrum of **1b** (Figure 3b, ^{13}C atom of Ala(1)). The two amide proton peaks of Aib can therefore be assigned to the trans (90%) and cis (10%) conformations of the Ala(1)-Pro peptide bond. The Ala(1) signal may be additionally split due to the trans and cis forms of urethane,[25,26] and the ratio of these three amide proton signals is 1:8:1, indicating that the main peak and one small peak are due to the trans Ala(1)—Pro peptide bond.

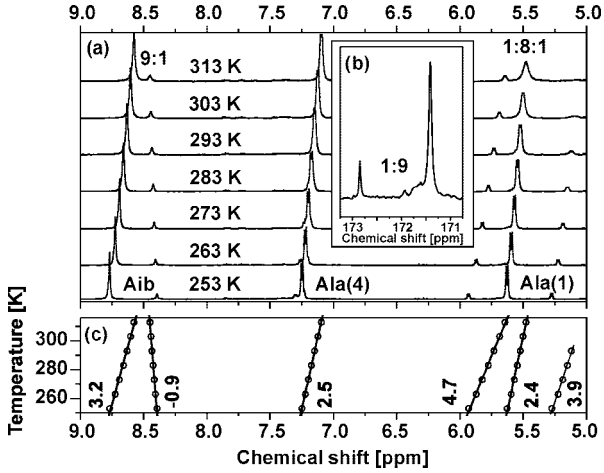

Figure 3. (a) ^{1}H NMR spectrum of thioxopeptide **1a** in CD_3CN in the NH-region at temperatures between 253 and 313 K. (b) The ^{13}C NMR peaks of the ^{13}C═O carbon (Ala(1)) of thioxopeptide **1b**. (c) Temperature-dependent chemical shift gradients (given in ppb/K) of the O═C—NH- and S═C—NH- protons, respectively.

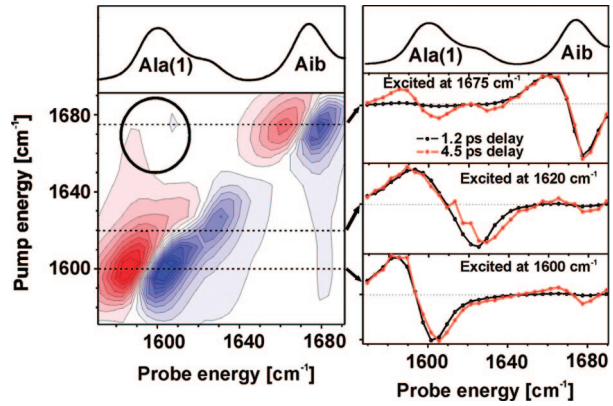

Figure 4. Left: 2D-IR spectrum of thioxopeptide **1b** at 293 K for a 1.2 ps time delay between pump and probe pulses with perpendicular polarization. Red corresponds to positive, blue to negative signals. The difference between two contour lines is 0.14 mOD. Right: Normalized cuts at 1600, 1620, and 1675 cm^{-1} for 1.2 (black) and 4.5 ps (red) time delays.

In Figure 3c the chemical shifts for the NH-protons are plotted as a function of temperature. The main NH-proton peaks of Ala(1) and Ala(4) shift with 2.5 and 2.4 ppb/K (ppb = parts per billion), which is significantly less than N-methylacetamide ($H_3CCONHCH_3$, 8.0 ppb/K) in the same solvent (N-methylthioacetamide $H_3CCSNHCH_3$ in acetonitrile shows a ^{1}H NMR-temperature dependence of 3.8 ppb/K. Therefore, the value for the SC-NH proton should not be compared to the OC-NH protons). Since the chemical shift of protons, which participate in an intramolecular H-bond, changes less as a function of temperature than that of protons that are fully exposed to the solvent,[26] this observation is consistent with the amide proton of Ala(4) acting as donor in a $i \rightarrow i + 3$ H-bond with the carbonyl oxygen of Ala(1) and can explain the double peak in the IR-spectrum of the isotope-labeled molecule. In addition, partial $i + 3 \rightarrow i$ H-bonding with Ala(1) as a proton donor is indicated by the ^{1}H NMR data. Indeed, in the FTIR spectrum the stretch band of the C═O group at 1745 cm^{-1} (Ala(4)) is broadened, which could be due to its acting as an H-bond acceptor. However, the very small spectral changes observed for the C═O stretch bands of Boc and Ala(4) upon photo isomerization of the thioamide bond (see below) do not support this hypothesis.

3. 2D-IR Measurements. In Figure 4 the 2D-IR spectrum of **1b** is shown. A narrow-band IR-pump pulse is scanned across the amide I region and selectively excites different modes to the first excited state (vertical axis). After a 1.2 ps time delay the pump pulse is followed by a broadband IR-probe pulse, which measures the pump-induced transmission changes (horizontal axis). Bleach and stimulated emission of the fundamental transitions lead to a negative signal (indicated by blue color) on the diagonal of the 2D-spectrum, whereas enhanced absorption (indicated by red color) is seen from the anharmonically shifted excited-state absorption. In addition, if two modes are coupled, the excitation of one leads to a shift in frequency of the other, resulting in a cross peak. The coupling strongly depends on the spatial and spectral distance between the two modes. The cross peak (encircled in Figure 4) between the modes of Ala(1) and Aib thus indicates spatial proximity of these two residues, in line with the presence of a $i \rightarrow i + 3$ H-bonded conformation.

For longer time-delays between the IR-pump and IR-probe pulse, excitation transfer between different modes takes place, and cross peaks grow at the cost of the diagonal peaks (this can be viewed as the analogue of an NMR-NOESY experiment). However, after a few picoseconds the energy should be allocated to all C═O modes in a peptide with only four residues. In addition, low-frequency modes are populated by the decay of the C═O stretch excitations. This too can give rise to apparent cross peaks, whose intensity thus becomes less sensitive to molecular structure. On the right-hand side of Figure 4 we compare horizontal cuts through the 2D-IR spectrum for time delays between pump and probe pulses of 1.2 ps (gray) and 4 ps (red), which were normalized to the diagonal signal. The upper cut on the right-hand side of Figure 4 clearly shows that population transfer takes place to Ala(1) when the band of Aib (1672 cm^{-1}) is excited initially (two negative bands are growing in at 1600 and 1625 cm^{-1}, with corresponding positive excited-state absorption signals). The reverse is observed when the bands at 1600 or at 1625 cm^{-1} are excited. However, there is no sign for population transfer between the modes underlying the bands at 1600 and 1625 cm^{-1}. This is a strong indication that these two bands are due to molecules in different conformations that do not interconvert on a picosecond time scale.

B. UV-pump/IR-probe Measurements. Changes in the mid-infrared spectrum of thioxopeptide **1a/b** after $\pi \rightarrow \pi^*$ excitation are shown for different time delays between the UV-pump and the IR-probe pulse in Figure 5. At early delay times, negative absorption is visible at the spectral positions of the vibrations of the initial trans species with positive absorption appearing red-shifted from these negative signals. Both negative and positive signals decrease for longer delays; however, the time-dependence is different at different spectral positions (see Figure 5c). As discussed in ref 23, the red-shift of all bands in the amide I region at early delays is in part due to anharmonic coupling to low frequency modes, excited by the excess laser energy (released upon fast relaxation from S_2 to lower-lying electronic states).[17] As a result of energy dissipation from the low frequency modes to the solvent, this red-shift almost completely decays on a 10−15 ps time scale for peptide units further away from the photoexcited moiety ($i + 3$). On the other hand, the C═O oscillators (i and $i + 2$), which are nearest neighbors of the thiopeptide unit and are sensitive to the different charge distribution in the electronically excited state, continue to be shifted at longer time delays. Indeed, after a nonexponential initial decay, the Aib ($i + 2$) signal decreases exponentially on a longer time scale of 240 ps. This decay is associated

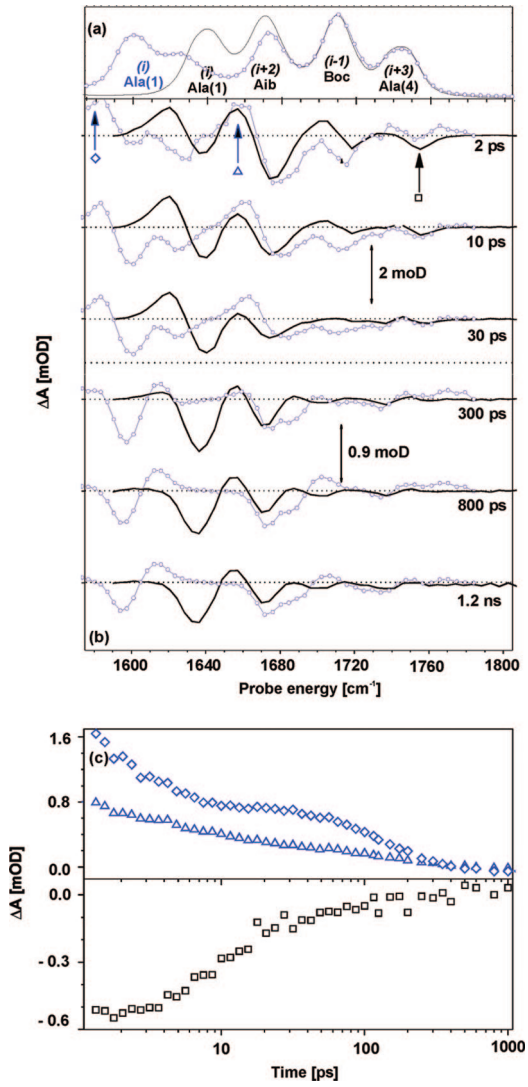

Figure 5. (a) FTIR absorption spectrum of thioxopeptides **1a** (black) and **1b** (blue). (b) Magic angle transient infrared difference absorption spectra of thioxopeptide **1a** (black) and **1b** (blue) after $\pi \rightarrow \pi^*$ excitation at different delays between the UV-pump and IR-probe pulse. (c): Time evolution of the C═O stretch mode absorption of Ala(1) (*i*, **1b**, blue diamonds), Aib ($i + 2$, **1a**, blue triangles) and of Ala(4) ($i + 3$, **1a**, black squares) mode absorption after $\pi \rightarrow \pi^*$ excitation. The signal shows the difference absorption signal at 1580 cm^{-1} for the C═O stretch mode absorption of Ala(1), at 1651 cm^{-1} for the C═O stretch mode absorption of Aib and at 1755 cm^{-1} for the C═O stretch mode absorption of Ala(4).

with relaxation into the electronic ground state, after the molecules have been trapped on the lowest-lying electronic excited state (see also refs 17 and 19). After 1 ns, the transient spectra no longer change. At this stage, positive absorption is due to molecules, which have isomerized into the cis conformation in the electronic ground state, whereas negative absorption is due to the loss of molecules in the trans conformation. The data for **1b** shows an decrease of the C═O stretch band of Ala(1) (amino acid *i*) at 1600 cm^{-1} and an approximately 50% smaller positive band at 1620 cm^{-1}. This is consistent with a $i \rightarrow i + 3$ H-bond, which is broken after the isomerization of the thioamide bond (see Figure 8). Both the red-shift and the oscillator strength of the initially H-bonded carbonyl stretch vibration are thereby reduced. Additionally, a blue-shift and apparent loss of oscillator strength can be seen for the C═O stretch absorption of Aib (amino acid at position $i + 2$), which belongs to the proton-donating peptide unit of the $i \rightarrow i + 3$ H-bond. Only a very small blue-shift is observed for the C═O stretch band of Boc and Ala(4), indicating that the terminal peptide units cannot be involved in strong intramolecular hydrogen bonds in the initial trans-state of the thioxopeptide.

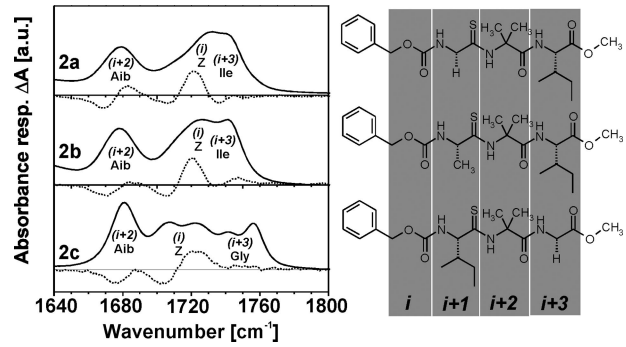

Figure 6. FTIR absorption spectra (solid lines) and FTIR difference absorption spectra (dashed lines) due to UV irradiation of the thioxopeptides **2a**−**c** in CD_3CN at 278 K.

C. Thioxopeptides 2a−c. ***1. Infrared Absorption.*** In Figure 6 the structures and FTIR absorption spectra (solid lines) of the three thioxopeptides **2a**−**c** are shown. The spectra of thioxopeptide **2a** and **2b** contain three bands in the amide I region, which have been assigned to the three C═O stretch modes using the same numbering of residues with respect to the thio substituted amino acid as for **1a** and **1b**. The spectrum of thioxopeptide **2c** shows two additional bands. The two bands at 1740 and 1756 cm^{-1} of thioxopeptide **2c** are assigned to different conformations of the C-terminal glycine due to the structural flexibility of this residue. On the other hand, the bands at 1707 and 1722 cm^{-1} are most probably both due to the C═O stretch mode of the Z-protection group (i) in different open and hydrogen-bonded conformations. It is very likely that the extended wings near 1700 cm^{-1} in the absorption spectra of the thioxopeptides **2a** and **2b** are also due to hydrogen-bonded Z-groups. This assignment is supported by the spectral changes observed upon isomerization of the thiopeptide bond.

2. Photoisomerization. FTIR difference absorption spectra of the thioxopeptides **2a**−**c** due to 248 nm irradiation ($\pi \rightarrow \pi^*$ excitation) are shown in Figure 6 (dashed lines). The thioxopeptides are almost exclusively in the trans conformation in thermal equilibrium, so positive absorption in the difference absorption spectra is due to the cis conformation of the thioxopeptides (isomerized species), while negative absorption is due to a loss of molecules in the trans conformation. Despite the distinct absorption spectra of **2a**−**c**, the absorption changes due to irradiation are very similar. All three molecules show a blue-shift of the Aib band near 1680 cm^{-1}, less absorption around 1705 cm^{-1}, and a strong positive signal near 1720 cm^{-1}. Only minor changes are observed in the spectral region of the ester group. The shape of the difference spectra near 1680 cm^{-1} corresponds to the negative derivative of the C═O stretch band of Aib. This indicates a blue-shift of the cis band with respect to the trans band that is small compared to the width of the absorption band. On the other hand, in the C═O stretch mode region of the Z-protection group (i) of **2c** there is a clear bleach of the 1707 cm^{-1} band at the cost of the 1722 cm^{-1} peak. This can be understood as a decrease in population of molecules with a hydrogen-bonded Z-protection group. The similar difference signals for **2a** and **2b** then indicate that these molecules, too, can exist in the trans-form in conformations with hydrogen-bonded C═O groups of the urethane, which give rise to the low frequency shoulder of the 1720 cm^{-1} absorption bands. Indeed, we have noted previously[22] that the signals arising from

TABLE 1: Time Constants Observed in UV-Pump/IR-Probe Measurements of the Photoisomerization Reaction after $\pi \rightarrow \pi^*$ Excitation (top) and for Thermally Activated Cis → Trans Relaxation in the Electronic Ground State at 278 K

thiopeptide	**2a**	**2b**	**2c**	**1a**
$\pi \rightarrow \pi^*$ ex., abs. in $i + 3$ region [ps]	~11	~12	~8	~14
$\pi \rightarrow \pi^*$ ex., abs. in $i + 2$ region [ps]	150	130	160	240
thermally activated relaxation [sec]	7.4	3.9	1.5	≤0.5[a]

[a] Below time resolution of FTIR spectrometer.

the breaking of intramolecular hydrogen bonds are dominant in the amide I IR difference spectra of thioxopeptides.

After irradiation at 248 nm is stopped, the system is out of equilibrium, and thermally activated cis → trans relaxation in the electronic ground-state takes place. The kinetics of this process have been measured by monitoring the decay of the irradiation-induced FTIR difference signal (dashed lines in Figure 6 at 278 K[33]). Single-exponential decay with time constants of 7.4, 3.9, and 1.5 s was observed for **2a**, **2b**, and **2c**, respectively (see Table 1 and Supporting Information for time traces). The back-reaction in the electronic ground-state was thus rate-determining for the formation of the photoequilibrium, which made it impossible to reliably determine the photoisomerization quantum yields via kinetic measurements. For thioxopeptides **1a** and **1b** the back-reaction was so fast that even at 278 K we could not accumulate a sufficient amount of photoproduct inside the FTIR spectrometer under irradiation conditions that did not damage the sample.

The transient spectra of thioxopeptide **2a** (black solid lines in Figure 7b) show the same features as those of **1** in Figure 5. An early red-shift of all C═O stretch bands, a fast decay (≈ 10 ps) of the ester signal, and a slower (150 ps) evolution of the final difference spectrum, characterized by a blue-shift of the bands of the nearest neighbors of the thioswitch, are all visible. Similar signals are observed for **2b** and **2c** (see Figure 7d for time traces and Table 1 for a comparison of the time constants). The generalized reaction scheme in Figure 8 illustrates the different steps in the photoisomerization process common to all thioxopeptides.

Transient spectra for **2b** and **2c** (blue and red lines with symbols) are plotted in Figure 7b for time delays of 2 ps and 1 ns. The 2 ps spectra have been scaled to superimpose the spectrally well-isolated Aib signals, in order to correct for different excitation densities, and the spectra after one nanosecond delay have been multiplied by the same factors. Because the C═O stretch band of Aib has a similar line shape in all three thioxopeptides, the similar amplitudes of the 1 ns signals indicate comparable quantum yields of isomerization for **2a**−**c**.[34]

D. Anisotropy and Structural Information. Information about angles between the UV-transition dipole moment and the different IR-transition dipole moments can be extracted from the anisotropy of the pump−probe data at very short delays, which is given by $r(t) = (S_{\parallel}(t) - S_{\perp}(t))/(S_{\parallel}(t) + 2S_{\perp}(t))$, where $S_{\parallel}(t)$ and $S_{\perp}(t)$ stand for the signal recorded with parallel and perpendicular polarization, respectively, of the UV-pump and IR-probe light. In Table 2 we list $r(t \rightarrow 0)$ for the spectrally sufficiently isolated vibrational Aib band ($i + 2$) and the approximate anisotropy (average of $r(t)$ over first 20 ps) for the amino acid at position $i + 3$ (see Supporting Information for time traces).

The measured anisotropies for Aib ($i + 2$) are very similar (−0.11 to −0.12) for the different thioxopeptides, whereas the ones for the amino acid at position $i + 3$ scatter more strongly (0.07−0.16). Amide I transition dipole moments are known to

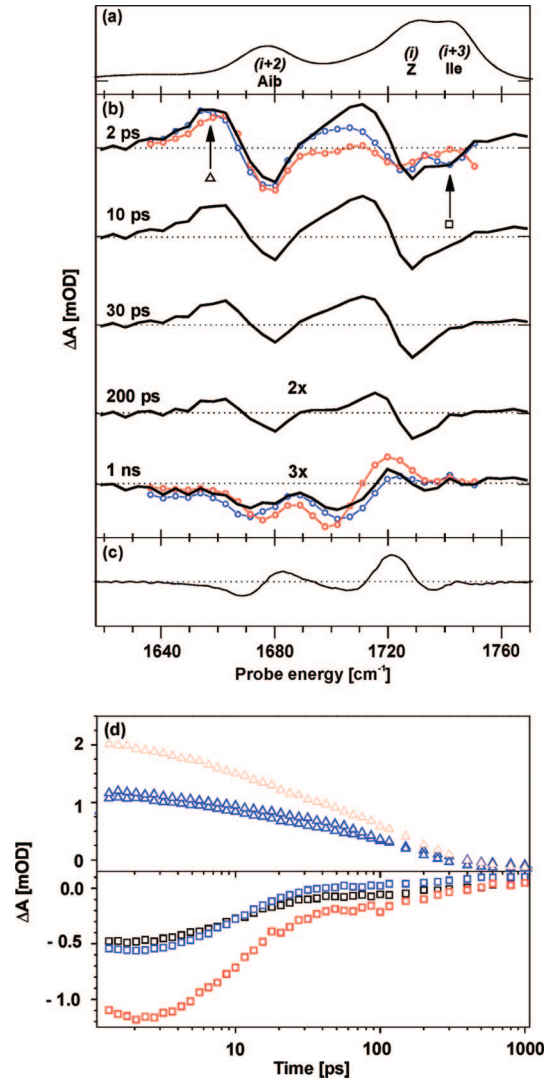

Figure 7. (a) FTIR absorption spectrum of thioxopeptide **2a**. (b) Magic-angle transient infrared difference absorption spectra of thioxopeptide **2a** (black), **2b** (blue), and **2c** (red) after $\pi \rightarrow \pi^*$ excitation with different delays between the UV-pump and IR-probe pulse. (c) FTIR difference absorption spectrum of thioxopeptide **2a**. (d) Time evolution of the C═O stretch mode absorption of Aib ($i + 2$, triangles) and of the amino acid at position $i + 3$ (squares) mode absorption after $\pi \rightarrow \pi^*$ excitation. The signal shows the difference absorption signal at 1658 cm^{-1} for the C═O stretch mode absorption of Aib and at 1742 and, respectively, 1751 cm^{-1} for the C═O stretch mode absorption of the amino acid at position $i + 3$ of thioxopeptide **2a** (black) and **2b** (blue), respectively, **2c** (red).

be well localized and can be approximated by a point dipole in the plane of a peptide unit forming an angle of 20° with respect to the C═O bond.[27,28] The UV transition dipole moment of the thiopeptide unit can be approximated by the one determined for NMTAA in connection with the ab initio calculations reported in ref 17. This transition dipole, too, lies in the (thio) peptide plane at an angle of 16° with respect to the C═S bond (≈40° with respect to the N−C bond,[29] see Figure 1). The measured anisotropies for thioxopeptide **2c** in solution can thus be compared with those expected for the crystal structure[21] (last column in Table 2). The good agreement for Aib suggests a conserved and very rigid -(SC−NH)-Aib- core structure. Indeed, Aib is known to define the structure of its adjacent moieties with very little margin. The differences for the amino acid at position $i + 3$ point to greater flexibility of the terminal group or a poor representation of the ester transition dipole moment

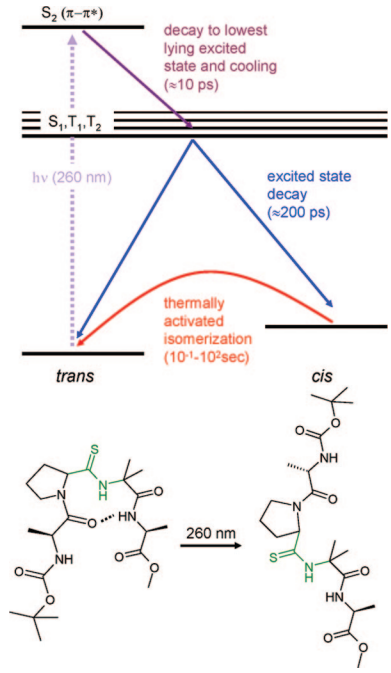

Figure 8. Top: Reaction scheme for the photo isomerization of thioxopeptides based on refs [17,19,18,23,11] and this work. Bottom: Schematic representation of the structural change of thioxopeptide **1a** after -Pro-ψ(SC—NH)-Aib- isomerization triggered by $\pi \rightarrow \pi^*$ excitation.

TABLE 2: Anisotropy Values between UV- and IR-Transition Dipole Moments (Only Residues $i + 2$ and $i + 3$) of the Thioxopeptides 1a and 2a−c

thiopeptide		**1**[a]	**2a**[a]	**2b**[a]	**2c**[a]	**2c**[b]
anisotropy	$i + 2$ (Aib)	−0.12	−0.11	−0.11	−0.12	−0.12
	$i + 3$	0.16	0.08	0.07	0.16[c]	0.35

[a] Determined by the time-resolved measurements. [b] Calculated from crystal structure.[21] [c] Band at 1756 cm^{-1}.

by the amide I mode of a peptide unit. Nevertheless, it is apparent from the comparison of the anisotropy data for the four molecules that the tails of the thioxopeptides are more flexible than their inner part.

IV. Discussion

The propensity of different thioxopeptides to form intramolecular hydrogen bonds has, in the past, been inferred from infrared absorption spectra in acetonitrile and CH_2Cl_2 solution.[30] In the nonpolar solvent distinct NH-stretch bands arise for H-bonded amide and thioamide units, and a dominant β-turn conformation was identified for the protected thioxopeptide Boc-Pro-Gly-ψ(SC—NH)-$NHCH_3$. In Boc-Pro-ψ(SC—NH)-Gly-$NHCH_3$, on the other hand, the thioamide proton was found to take part in a H-bond with the neighboring carbonyl group, forming a competing C_5 ring structure.[30] Since thioamide protons are stronger hydrogen bond donors than amide protons, a perturbation of the β-turn conformation of **1** should be considered. Indeed, the FTIR spectrum of the precursor molecule during synthesis, Fmoc-Pro-ψ(SC—NH)-Aib-Ala-OMe exhibits, in chloroform, a band due to an H-bonded thioamide proton at 3260 cm^{-1}, indicating the presence of a C_7 ring structure most likely with the Fmoc carbonyl group acting as the H-bond acceptor (see Supporting Information for spectra). This NH-stretch band, however, transforms into a weak tail in the absorption spectrum of **1**, and we infer that the red-shifted carbonyl stretch band of Ala(1) at 1600 cm^{-1} is entirely due to the $i \rightarrow i + 3$ H-bond in the β-turn conformation. Both the polarization-dependent UV-pump/IR-probe and the IR-pump/IR-probe data conform with this structure. In addition, the spectral changes observed upon photoisomerization of the thiopeptide are much more specific for identifying the intramolecular hydrogen bond in the dominant equilibrium trans-conformation than the steady state absorption and temperature-dependent NMR data. Assuming, based on the 1 ns difference spectrum in Figure 5, a 1.5 times larger oscillator strength for hydrogen-bonded Ala(1) band with respect to the 1625 cm^{-1} absorption, we estimate that approximately 60−70% of all molecules adopt the β-turn conformation in acetonitrile solution at room temperature.

In a very similar oxopeptide Boc-Cys-Pro-Aib-Cys-OMe this β-turn structure has been stabilized by a disulfide bridge between the two cysteine sidechains.[31] Opening of a part of these turn structures after light-cleavage of the disulfide bond led to a new equilibrium between hydrogen-bonded and nonhydrogen-bonded conformations on a 200 ps time scale.[32] The final conformational distribution in that experiment may be regarded as similar to the starting distribution of thioxopeptide **1** before the photoisomerization of the thioamide bond, which perturbs this equilibrium.

In the electronic ground state, where there is a significant barrier to (thermally activated) cis → trans isomerization, the thermodynamically stable trans-conformation is re-established significantly faster (subsecond at room-temperature) in the four molecules studied here than in non-Aib-containing thioxopeptides (several minutes).[11] Furthermore, these isomerization rates strongly increase with the size of the residue of the thiosubstituted amino acid at position $i + 1$ (with the rigid proline residue showing even shorter times than isoleucine). The conformational preference induced by the Aib residue and steric interaction between the side chain of the thiosubstituted residue $i + 1$ and Aib thus seems to strongly destabilize the molecules in the cis conformation of the thioamide bond. The cis → trans relaxation rates as a function of temperature, which have been reported for a series of thioxopeptides in ref 11, suggest that entropic contributions to the barrier height, rather than enthalpic differences, are primarily responsible for this destabilization. The much faster rates we observe for the molecules containing the -ψ(SC—NH)-Aib- motif may thus be a consequence of their reduced conformational flexibility. Note that the cis → trans relaxation rates also increase with the thermodynamic stability of H-bonded conformations in the ground-state of thioxopeptides **2a−c**.

On the other hand we have measured very similar time constants for the photoisomerization reaction of all four thioxopeptides containing the -(SC—NH)-Aib- moiety. The photoisomerization times are also in agreement with the excited-state lifetimes of a variety of other thioxopeptides of different sizes and different amino acid residues next to the thiopeptide unit.[19] In addition, in all thioxopeptides investigated so far, quantum efficiencies of photoisomerization are significant (10−40%) and differ much less than the ground-state isomerization time constants (although an accurate determination of quantum efficiencies is difficult in the present case, where the ground-state relaxation is very fast). These observations call for some force driving trans → cis photoisomerization in apparent contradiction with ab initio excited-state energy surfaces, which are essentially flat along the torsional coordinate.[17] However, at the moment when a molecule relaxes back to the electronic ground-state, a strong driving force is predicted. The region in

conformational space where the ground-state is reached and from which the cis state can be formed via a driven motion[17] is apparently accessible via diffusive motion along a thioamide single bond in the excited state, without strong interference from side chains or the rest of the peptide backbone. The absence of a strong driving force in the excited-state is also consistent with our observation of a relatively long-lived red-shift of the initially hydrogen bonded Ala(1) C═O stretch band. Its persistence indicates a relatively long lifetime of the $i \rightarrow i + 3$ hydrogen bond after photoexcitation, which would be highly unlikely in the case of a forced isomerization of the thiopeptide unit on the excited-state surface.

V. Conclusion

The ability to photoisomerize monosubstituted thioxopeptides with sterically ambitious residues next to the photo switch moiety promises further applications of thioxopeptides. Aib-containing sequences, which efficiently stabilize β-turns and 3_{10}-helices, can be synthesized comfortably using the azirine/oxazolone-method and form a very rigid inner part of the molecules. An appropriate choice of the thio-substituted residue then allows one to tune the time-constant for thermally activated cis → trans isomerization in the electronic ground-state to the subsecond regime, without dramatically affecting the trans → cis photoisomerization efficiency. As a building block of a larger sequence, the -Pro-ψ(CS—NH)-Aib- motif introduced here could, for example, be used to gain photocontrol over the formation of an essentially native β-sheet structure.

The current experiments were carried out in acetonitrile because of better solubility and in order to enhance intramolecular hydrogen-bond formation in very short sequences. Additional residues are likely to increase secondary structure propensity, making it possible to also work in water with a well-defined initial conformational distribution. On the basis of our findings for the isolated thioamide switch,[18] we do not expect any significant changes in timescales and isomerization efficiencies when using aqueous solvent in the future.

Our current picture of the mechanism by which efficient photoisomerization is possible in a large number of monosubstituted thioxopeptieds, involves a diffusive torsional motion about a thioamide single bond during the ≈200 ps lifetime of the lowest lying electronically excited state, followed by strongly driven motion in the electronic ground state. Further theoretical work is now needed in order to establish if a sufficiently large region of conformational space can be sampled by the photo-excited peptides within 200 ps to reach the favorable conical intersections or intersystem crossing points, which, in the gas phase, have been predicted to lead to the cis photoproduct.

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Supporting Information Available: A detailed description of the synthesis of **1a/1b**, their FTIR spectra in chloroform and temperature-dependent absorption spectra in CD_3CN are provided. Spectra and time traces underlying the data in Table 1 (thermally activated ground-state isomerization of **2a**−**c**) and Table 1 (anisotropy) are also shown. This information is available free of charge via the Internet at http://pubs.acs.org.

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(33) The relaxation times at room temperature of all four thioxopeptides lie below the time-resolution of our conventional FTIR-spectrometer.
(34) In addition to isomerization, the pump laser also causes small changes in sample temperature (increase of ≈0.5 K in the excited volume) which can also affect the infrared absorption spectrum.[23] For **1a,b**, temperature-induced changes in the FTIR spectra were found to be very different from the nanosecond pump−probe signal (see Supporting Information); however, for **2a**−**c**, a temperature contribution to the transient spectra cannot be excluded.

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