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A New Tool in Peptide Engineering: A Photoswitchable Stilbene-type β-Hairpin Mimetic

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Abstract: Peptide secondary structure mimetics are important tools in medicinal chemistry, as they provide analogues of endogenous peptides with new physicochemical and pharmacological properties. The development, synthesis, photochemical investigation, and conformational analysis of a stilbene-type β-hairpin mimetic capable of light-triggered conformational changes have been achieved. In addition to standard spectroscopic techniques (nuclear Overhauser effects, amide temperature coefficients, circular dichroism spectroscopy), the applicability of self-diffusion measurements (longitudinal eddy current delay pulsed-field gradient spin echo (LED-PGSE) NMR technique) in conformational studies of oligopeptides is demonstrated. The title compound shows photoisomerization of the stilbene chromophore, resulting in a change in solution conformation between an unfolded structure and a folded β-hairpin.

Keywords: beta-hairpin · peptidomimetics · photochemistry · self-diffusion · stilbene

Introduction

Molecular switches are compounds capable of existing in two or more interconvertible conformational states.[1] External switching between these isomers may be driven by pH change, chemical reactions, complexation, electron transfer, heat, or magnetic or electric fields, or it may be triggered by irradiation at a selected wavelength.[2] The development of such photoresponsive systems is of outstanding interest because they have considerable potential for application as new tools in biomedical engineering.[3] Incorporation of a molecular switch into bioactive compounds may allow external modulation of their biological effect: it may, for example, enable photoactivation of a prodrug, in a manner resembling the bioactivation of vitamin D,[4] photoactivation of a drug in a specific area in the human body, or light-triggered association/dissociation of a ligand–receptor complex. As a key step towards the development of such compounds, we present here a photoswitchable, stilbene-based peptidomimetic and its conformational analysis.

The β-hairpin motif is involved in numerous vital physiological processes and pathological disorders. Because the biological activities of some β-hairpins[5] have been shown to be correlated with the thermodynamic stabilities of their folded conformations,[6] a β-hairpin mimetic incorporating a stilbene moiety should be an attractive candidate for a photoswitchable peptidomimetic. Such a mimetic should allow externally triggered interconversion between a bioactive β-hairpin and a bioinactive nonhairpin conformation. The incorporation of a diazobenzene dipeptide mimic into cyclic[3,7] and acyclic[8] peptides was recently reported, but the hairpin-inducing ability of this turn mimic appeared to be limited.[7b] In addition, diazobenzenes are in many ways inferior to stilbene derivatives in that they undergo thermal *cis–trans* isomerization and are sensitive to reducing agents, whilst the separation of their isomers is also often complicated, if not impossible.[7b,8] At present, no attempts at incorporation of stilbene derivatives into peptides have been reported. Because of their greater chemical stability, however, they would be attractive alternatives to azobenzenes.

Results and Discussion

Here we present the development of a stilbene-type peptidomimetic capable of light-triggered conformational changes between an unfolded structure and a folded β-hair-

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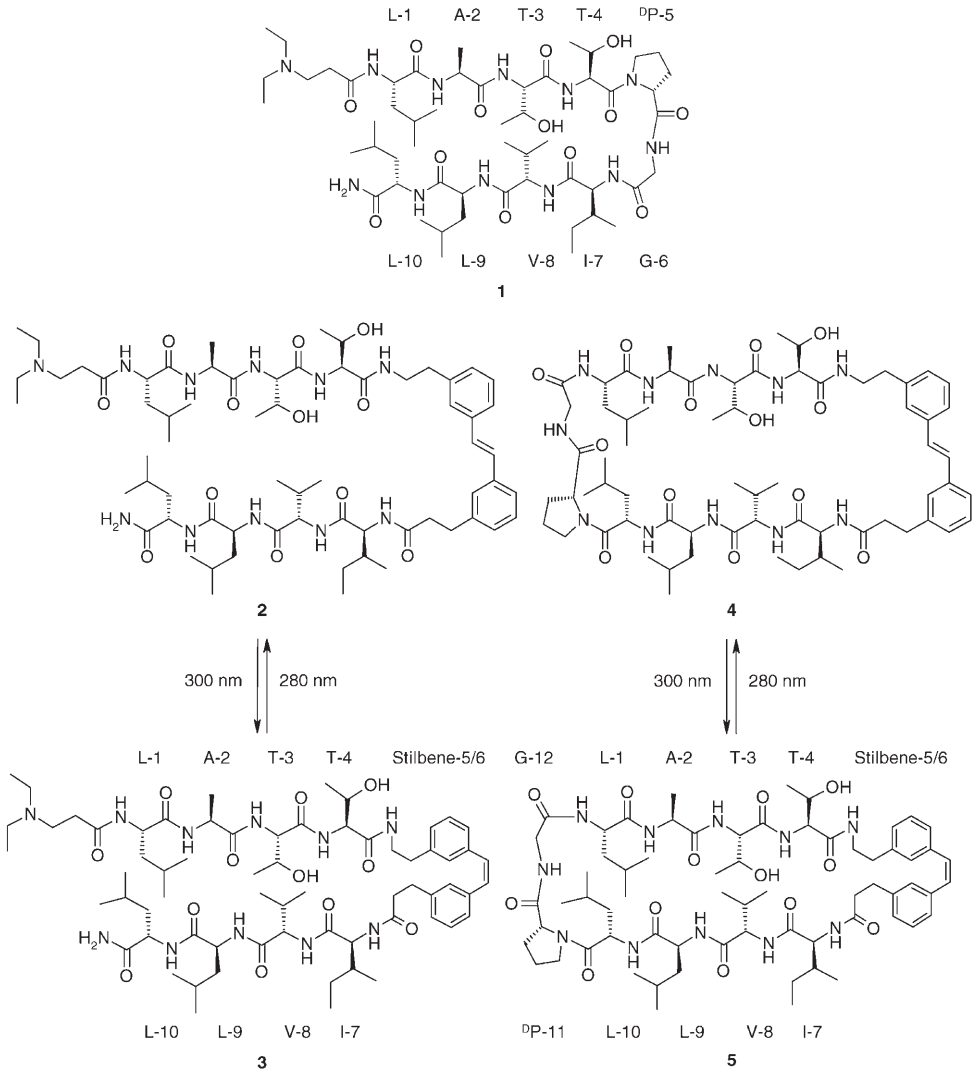
pin form. In this model study, the *trans* isomers of a cyclic and an acyclic peptidomimetic—cyclo(Leu-Leu-Val-Ile-stilbene-Thr-Thr-Ala-Leu-Gly-^{D}Pro) and Leu-Leu-Val-Ile-stilbene-Thr-Thr-Ala-Leu-X—were prepared by combination of solution-phase and solid-phase methodologies (X = diethylaminoacetyl; Scheme 1) and their *cis* isomers were obtained by subsequent photoisomerization. The amino acid strands of the investigated compounds were extracted from the S4 region of the TATA-box binding protein, and are known to fold into β-sheets in their native environments.[9] Surprisingly, no attempts at application of these amino acid strands in model hairpin studies have yet been reported, although their sequences are very likely to provide stable β-hairpin conformations, as aliphatic β-branched amino acids are known to promote the formation of hydrophobic clusters,[10] and so are frequent constituents of the strand segments of native β-hairpins.[11] For comparison, the nonswitchable analogue Leu-Leu-Val-Ile-Gly-^{D}Pro-Thr-Thr-Ala-Leu-X was also prepared by standard solid-phase peptide synthesis (SPPS). A ^{D}Pro-Gly dipeptide incorporated into **1** is a well-known β-turn inducer,[12] so this peptide was designed to fold into a β-hairpin. The acyclic (**2** and **3**) and cyclic (**4** and **5**) peptidomimetics incorporating a photoswitchable stilbene unit were expected to allow externally triggered folding and unfolding through irradiation at selected wavelength(s). In addition, diethylaminoacetyl tails were also attached to the N termini of all but the cyclic peptides **4** and **5** to increase their solubility in polar solvents.[13]

Photochemistry: Isomerizations of **2**–**5** were carried out in dimethyl sulfoxide by selective use of irradiation at the absorption maxima ($\lambda_{max} = 300$ or 280 nm)[14] of the *trans*- or *cis*-stilbene double bonds. In the case of **2**, the maximum percentage of *cis* isomer **3** at the photostationary state was reached after 3 h of irradiation ($\lambda = 300$ nm) and was 63%, as determined by ^{1}H NMR spectroscopy. For **4**, the photo-



Scheme 1. The structures of β-hairpin mimetic **1**, together with its open-chain (**2**, **3**) and cyclic (**4**, **5**) photoswitchable analogues. Amino acid building blocks and photochemical transitions are indicated.

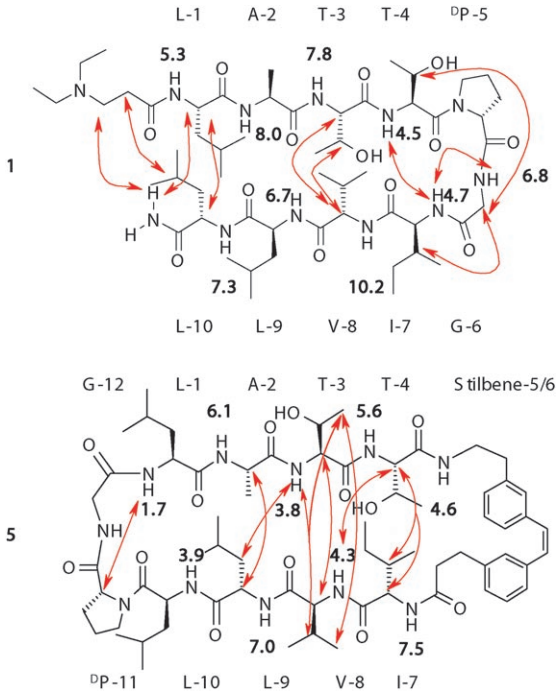
stationary state (80% *cis* isomer **5**) was reached after 90 min of irradiation at $\lambda = 300$ nm. Isomerization of the *cis* isomer **5** at $\lambda = 280$ nm resulted in 16% of the *trans* compound **4**.

Attempts to separate the *cis* and *trans* isomers by preparative reversed-phase HPLC were successful in the case of compounds **4** and **5**, so—in contrast with similar studies on azobenzene derivatives[15]—the *cis* peptidomimetic **5** was analyzed separately from its *trans* isomer, subsequent to their separation. Because of the lack of separation of the acyclic compounds, the conformation of **3** was successfully investigated in the *cis–trans* mixture obtained after isomerization of **2**.

Conformational analysis by NMR spectroscopy: The most reliable and extensively employed tool used today for exploration of peptide solution conformations is without doubt the detection of nuclear Overhauser effects (NOEs).[16] The observation of nonsequential NOEs hence provides the strongest evidence for the three-dimensional solution structure of a compound, and so conformational analysis was first performed with standard NMR techniques in water, methanol, and dimethyl sulfoxide.[13] As expected, interstrand NOEs observed in the solutions of compound **1** in methanol and dimethyl sulfoxide were characteristic of a β-hairpin (Scheme 2). However, the absence of such NOEs in aqueous solution suggests that folded conformations observed in methanol and dimethyl sulfoxide have low thermodynamic stability. No NOEs between nonadjacent residues were observed in the cases of compounds **2**, **3**, and **4**, thus clearly indicating unfolded solution structures. It should be mentioned, though, that the NOESY spectra obtained for **4** showed a few indications of the formation of a ^{D}Pro-Gly β-turn. In contrast with its open-chain analogue **3**, interstrand NOEs observed in the case of the cyclic peptidomimetic **5** provided strong evidence of its folding into a β-hairpin conformation (Scheme 2).

The absence of any aggregation phenomena was revealed by the concentration independence of the amide chemical shifts, as well as by the observation of sharp ^{1}H NMR signals for all investigated compounds. No chemical shift change or line-broadening was observed over a period of three weeks. In addition, pulsed-field gradient spin echo (PGSE) NMR translational diffusion studies performed with dimethyl sulfoxide confirmed that all compounds were present in a monomeric, disaggregated form.

Temperature coefficients ($\Delta\delta/\Delta T$) offer a qualitative measure of the involvement of amide protons in intramolecular hydrogen bonding and thus an additional insight into peptide secondary structures.[13a] As expected, the temperature coefficients obtained for the solution of compound **1** in dimethyl sulfoxide (Table 1) were consistent with a folded β-hairpin structure in which the amide protons of Leu-9 and Ile-7 are intramolecularly hydrogen bonded and Thr-4 is in equilibrium between hydrogen-bonded and nonbonded states.[17] The $\Delta\delta/\Delta T$ values obtained for **1** in methanol imply that the folded conformation has low thermodynamic stability and exists in exchange with unfolded structures in competitive solvents, an observation in good agreement with the NOE studies performed with water solutions (Scheme 2). Moreover, the alternating amide temperature coefficients of the *cis* isomer of the cyclic peptidomimetic **5** are typical of a folded β-hairpin structure in which the amide protons of Leu-1, Thr-3, Stil-



Scheme 2. NMR evidence for folded β-hairpin conformations in compounds **1** and **5**. The building blocks, the observed amide temperature coefficients (bold), and interstrand NOEs (arrows) of **1** and **5** in methanol are indicated.

Table 1. The amide proton temperature coefficients $\Delta\delta_{NH}/\Delta T$ [ppb K^{-1}] obtained in dimethyl sulfoxide and methanol.[a]

	1		**2**		**3**		**4**		**5**	
	DMSO	MeOH	DMSO	MeOH	DMSO	MeOH	DMSO	MeOH	DMSO	MeOH
L-1	3.4	5.3	3.6	4.5	5.7	4.5	7.4	6.7	5.4	1.7
A-2	5.0	8.0	5.2	4.7	7.5	4.7	8.8	8.7	4.0	6.1
T-3	4.0	7.8	3.6	3.7	8.2	3.7	9.9	6.7	4.5	3.8
T-4	3.2	4.5	2.2	2.3	5.9	2.3	4.4	4.3	3.5	5.6
X-6[b]	2.6	6.8	3.8	3.9	5.7	3.9	3.9	2.6	3.9	4.6
I-7	1.6	4.7	4.1	4.2	7.8	4.2	4.4	2.8	5.6	7.5
V-8	5.5	10.2	5.2	5.1	9.7	5.1	5.5	7.9	4.0	4.3
L-9	2.7	6.7	3.8	3.7	7.3	3.7	5.5	6.3	4.5	7.0
L-10	4.3	7.3	3.6	4.2	8.1	4.2	10.7	5.7	4.9	3.9

[a] Temperature coefficients obtained as values $(\delta_{T,high}-\delta_{T,low})/(T_{high}-T_{low})$ that are negative numbers, but are reported as positive values in accordance with accepted literature conventions.[13a] [b] X-6 = G-6 or Stilbene-5/6.

bene-5/6, Val-8, and Leu-10 are intramolecularly hydrogen bonded (Scheme 2). It should be noted that the $\Delta\delta_{NH}/\Delta T$ values for this compound gradually increase in the direction of the stilbene linker, indicating the inferior turn-inducing properties of the artificial dipeptide mimic in relation to the ^{D}Pro-Gly sequence. In agreement with the NOESY and ROESY studies, the high $\Delta\delta_{NH}$ values observed for the solutions of the *trans* isomers of the acyclic and cyclic mimetics **2** and **4** and the acyclic *cis* isomer **3** were indicative of unfolded structures. The low temperature coefficients generally observed for the NH of Thr-4 might be due to the formation of a six-membered ring through hydrogen bonding to the hydroxy group of Thr-3.

The magnitude of $^{3}J_{NH,H\alpha}$ is known as an easily measurable parameter furnishing further indications of backbone conformation.[16] For compounds **2**–**4** the $^{3}J_{NH,H\alpha}$ coupling constants fall into the ranges typical for random coil ($\approx$6–7 Hz) or extended ($\approx$9 Hz) conformations, indicating the presence of a mixture of interconverting conformations in solution. The largest coupling constants indicative of extended strands were observed for peptide **1** and **5**.[14] It should be emphasized, though, that coupling constants in themselves are only indicators of—and do not prove any—peptide secondary structure.

Amino acid proton chemical shifts are also known indicators of peptide overall conformation.[18] Residues in β-strands or in extended conformations thus have higher chemical shifts (0.1–0.6 ppm) than those participating in random coils, while amino acids in α-helices and β-turns experience the opposite tendency and have low δ values. The observed chemical shift differences of the NH and H_α protons in corresponding amino acids of peptides may thus reveal folding tendencies. The chemical shifts of peptidomimetics **2** and **3** are similar and hence indicate that *cis–trans* isomerization does not induce folding or unfolding of the acyclic stilbene-containing peptide.[14] In constrast, the chemical shifts of the amide and α-protons, as well as the $^{3}J_{\alpha H,NH}$ coupling constants of most of the amino acids of *cis* isomer **5** of the cyclic peptidomimetic are higher than those found for its *trans* isomer **4**,[14] so these changes suggest a photoisomerization-triggered folding process in the cyclic peptidomimetic. Again, we would like to stress that chemical shifts, unlike observation of nonresidual NOEs, are in themselves only indicative but not conclusive regarding secondary structures. It should be noted, though, that our $^{3}J_{\alpha H,NH}$ and δ data are in good agreement with the observed NOEs and $\Delta\delta_{NH}/\Delta T$ values.

Molecular modeling: To model the preferred conformations of our peptides, Monte Carlo conformational searches followed by conjugate gradient minimizations were performed. As the solution structures of the folded conformations of our peptidomimetics were observable through NOEs and the established structures were supported by a number of other NMR parameters, we felt obliged to take advantage of the way constraint-processing techniques and conformational search techniques complement each other.[19] Thus, through the use of NMR constraints derived from NOE data obtained for solutions of the compounds in methanol (Scheme 2) the reliability of the calculation output was considerably improved and the processor time was radically decreased in relation to initial unrestricted calculations. The computations resulted in 402 (**1**), 204 (**4**), and 302 (**5**) structures within 3 kcal mol^{-1} of the global minima. The ten lowest-energy conformations for the reference compound **1** and the *trans* (**4**) and *cis* (**5**) isomers of the photoswitchable cyclopeptide are depicted in Figure 1 (top, middle, and bottom, respectively). Our computations indicate that the folded geometric arrangement is strongly preferred in the cases of **1** and **5**, whereas **4** favors unfolded conformations and hence, in its lack of stabilizing intramolecular hydrogen bonds and hydrophobic interactions, is notably more flexible than its folded analogues.

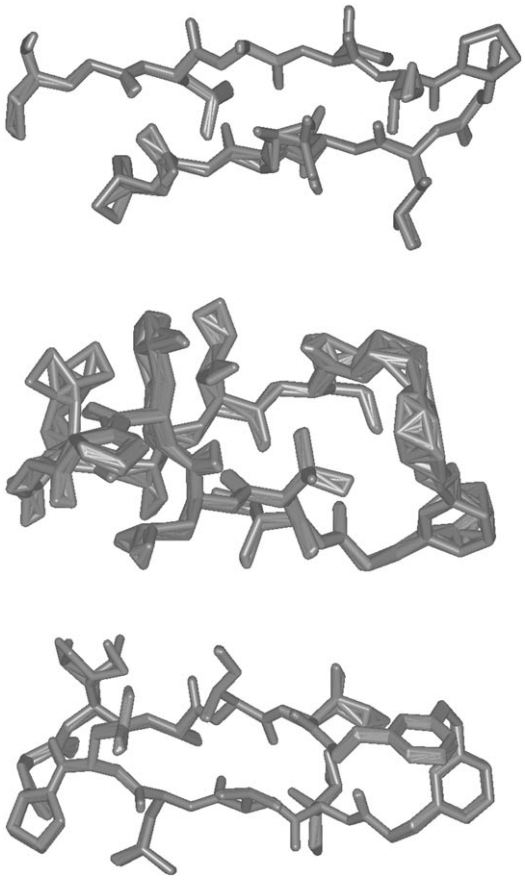


Figure 1. Representative solution conformations of **1** (top), **4** (middle), and **5** (bottom). Overlaid backbones of the ten lowest-energy conformations resulting from NOE-restrained Monte Carlo conformational search followed by conjugate gradient minimization are shown.

Circular dichroism (CD): Circular dichroism spectroscopy, allowing the investigation of overall solution conformations, is a further commonly applied tool in peptide chemistry. Compound **1** exhibited a negative band indicative of a β-hairpin conformation at 220 nm (Figure 2). The observed low ellipticity and a second minimum at approximately

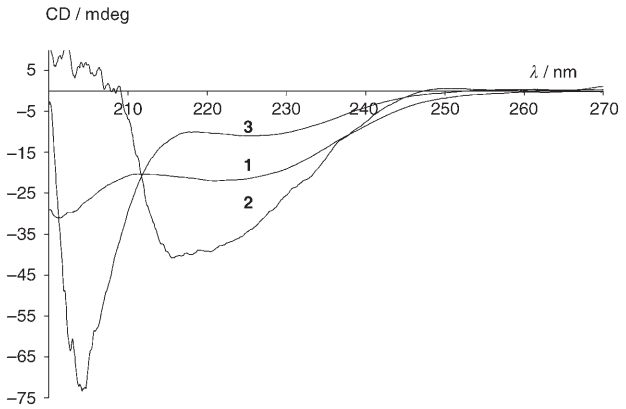


Figure 2. CD spectra obtained with samples in methanol. The CD spectrum of **3** was obtained by subtraction of the absorbance of the *trans* isomer **2** from the CD spectrum of the photostationary state.

200 nm indicate that the folded conformation is in equilibrium with unfolded structures. The CD of **2** showed a broad negative band at 214–220 nm, a weak positive absorption centered at 280 nm, and a negative band at 300–350 nm. Compound **3** showed two weak negative absorptions at 209 and 217–224 nm. Although the stilbene chromophore does not interfere with the amide region of the spectrum, because it has its main absorption region at 300–350 nm,[20] the obtained spectra are difficult to interpret in terms of folding because of the lack of CD data for similar compounds. The CDs of **4** and **5** confirm that the ^{D}Pro-Gly sequence functions as a turn inducer independently of the geometry of the stilbene double bond (Figure 3). Upon *trans* to *cis* isomeri-

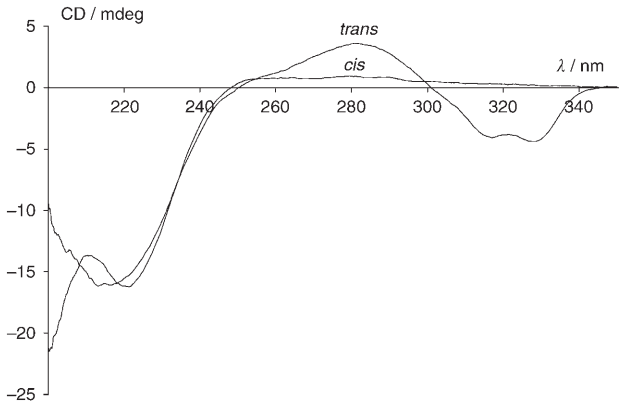


Figure 3. The CD spectra of the cyclic photoswitchable mimetics **4** (*trans*) and **5** (*cis*) in methanol.

zation the negative amide band shifts from 214 to 220 nm, a change that may be indicative of a β-turn to β-hairpin transition. We would like to stress that, although the obtained spectra are difficult to interpret, they are in good agreement with the single available report for CD investigation of stilbene derivatives,[20] and the observed change in the chiroptical features is in itself an indicator of a conformation alteration upon *cis–trans* isomerization of **4** and **5**. The results of the CD investigation of **1–5** are hence in good agreement with the conclusions drawn from NMR studies.

Translational self-diffusion measurements: Translational self-diffusion measurements using pulsed-field gradient spin echo (PGSE) NMR methods are known tools in structural investigation of peptides and proteins, as the translational diffusion coefficient (D_t) is related to the mass of a compound through its hydrodynamic radius.[21] Diffusion measurements are therefore commonly applied in order to draw conclusions about monomeric or oligomeric states, but have in a few cases also been used to follow conformational changes in polypeptides.[22] In spite of the simplicity and rapidity of this technique, its use for conformational investigation of small peptides is extremely rare in the literature. We anticipated that the light-triggered conformational change of our cyclic peptidomimetic between β-hairpin and random coil forms might be reflected in different diffusion behavior, so the self-diffusion coefficients were measured by use of the LED-PGSE[23] pulse sequence in [D$_6$]DMSO at 25 °C and were determined as 1.4×10^{-6} (*trans* isomer **4**) and 1.8×10^{-6} cm^{2}s^{-1} (*cis* isomer **5**).[24] For comparison, we estimated the diffusion coefficient of a peptide of comparable molecular weight by the theoretical method of Gräslund et al.[25] This model is applicable for prediction of the translational diffusion coefficient of an aqueous solution of a monomeric, linear, random-coil-forming peptide of known molecular mass. The theoretical prediction estimated somewhat slower diffusion for our peptide than experimentally observed: $D_t^{predicted}=2.6\times10^{-6}$ cm^{2}s^{-1}. If allowance is made for the differences between the conditions of the prediction and the experiments—that is, that the measurements were performed on a structured, cyclic peptide in dimethyl sulfoxide—the calculated diffusion coefficient is in good qualitative agreement with those observed experimentally. In addition, the translational self-diffusion coefficients were also estimated by use of the program HYDROPRO,[26] developed for globular proteins by Carrasco et al. The hydrodynamic modeling predicted a coefficient of 1.1×10^{-6} cm^{2}s^{-1} for both structures depicted in Figure 1 (**4** (middle) and **5** (bottom)). The diffusion coefficients estimated by the two theoretical methods are thus not identical with the experimentally determined values, but are both of comparable magnitude, hence confirming the absence of any aggregation for the investigated solutions. Furthermore, in the case of oligomerization of the β-hairpin-forming *cis* isomer, a decrease in the diffusion coefficient relative to the nonhairpin *trans* isomer would be expected,[27] rather than the observed increase. The experimentally observed small, but still significant, increase in the diffusion coefficient upon photoisomerization of the *trans* isomer may thus be an indicator of a decrease in the compound's hydrodynamic size.[25] Consequently, the higher diffusion coefficient of the folded *cis* isomer may originate from lower solvent accessibility of its intramolecularly hydrogen-bonded amide protons, a factor also indicated by low $\Delta\delta_{NH}/\Delta T$ values. The amide protons of

the unfolded *trans* isomer are likely to be hydrogen bonded to solvent molecules, as previously indicated by the high $\Delta\delta_{NH}/\Delta T$ values, so the intermolecular NH to DMSO hydrogen bonds may increase the effective size of the peptidomimetic. In parallel, the good hydrogen-bond-accepting properties of dimethyl sulfoxide are known to cause a radical decrease in the diffusion coefficient of water molecules.[28] However, the interpretation of the observed alteration in diffusion properties at the molecular level is rather difficult, because, unlike in the case of water,[29] there is very little experience of the behavior of solvent layers in dimethyl sulfoxide,[3] whilst the properties of residual water in dimethyl sulfoxide are also currently not well understood.[31] The diffusion coefficients for all investigated compounds are summarized in Table 2.

Table 2. Experimentally observed and calculated translational diffusion coefficients of the investigated peptidomimetics.

Compound	M_r [g mol^{-1}]	D [cm^{2}s^{-1}]	Predicted D [cm^{2}s^{-1}][25]
1	1122	0.9×10^{-6}	2.8×10^{-6}
2	1246	0.7×10^{-6}	2.6×10^{-6}
3	1246	0.7×10^{-6}	2.6×10^{-6}
4	1255	1.4×10^{-6}	2.6×10^{-6}
5	1255	1.8×10^{-6}	2.6×10^{-6}

As expected, the D_t values for peptides **1**–**3** were significantly lower than those of **4** and **5**, as these compounds are linear and thus have larger hydrodynamic size than their cyclic analogues. Moreover, in agreement with theory,[21] the diffusion coefficients of the linear compounds followed the inverse order of molecular weight. The identical diffusion coefficients of **2** and **3** agree well with our hypothesis, as their conformational investigation with standard NMR tools clearly indicated that both isomers were present in solution as ensembles of interconverting unfolded structures. In summary, the advantage of translational self-diffusion measurements in peptide conformational analysis is simplicity and rapidity. It should be noted, though, that diffusion coefficients, like CD spectra, provide only limited information and may only indicate the overall solution conformation of a compound, but cannot be interpreted in terms of detailed site-specific information.

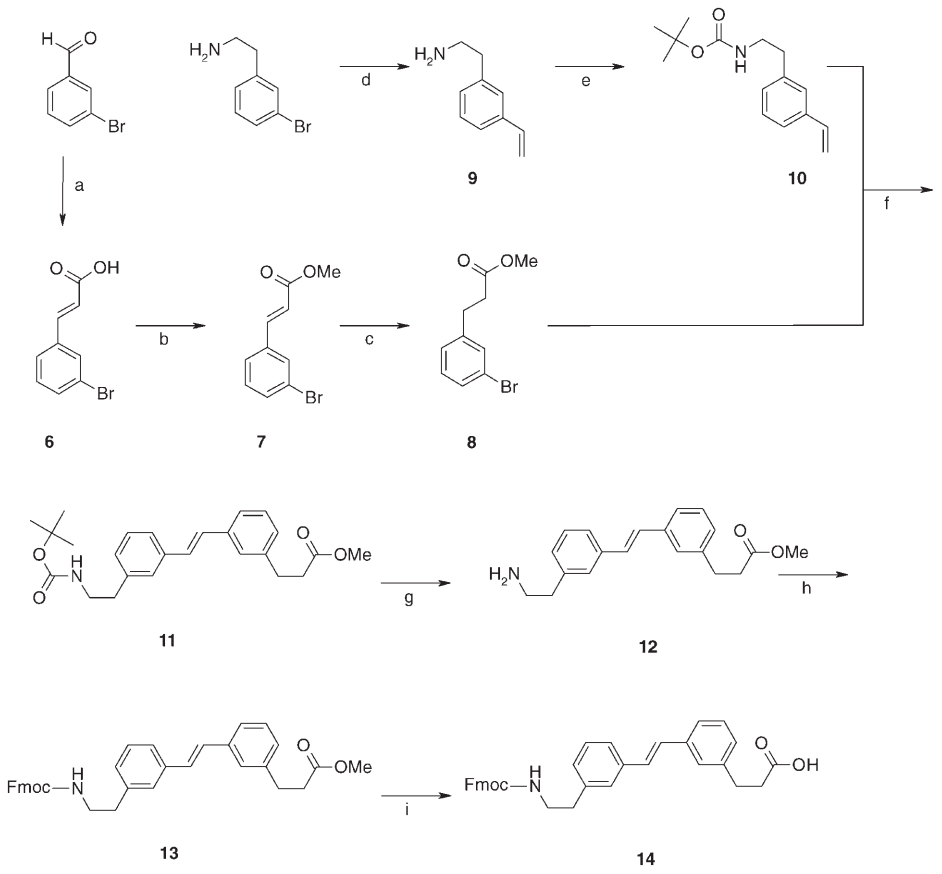
Conclusion

We have demonstrated that incorporation of a stilbene-type dipeptide mimic into a cyclic β-hairpin allows for light-triggered switching between different peptidomimetic conformations. Structural changes established by standard NMR techniques (NOEs, temperature coefficients) and indicated by CD measurements demonstrated folded β-hairpin and unfolded conformations for the *trans* and *cis* isomers, respectively, of the presented cyclic stilbene-containing peptide analogue. The comparable acyclic stilbene-type peptidomimetic, however, did not fold into a β-hairpin as its *cis* isomer (**7**), most possibly for the following entropic reasons: 1) high flexibility of the $(CH_2)_2$ linkers of the incorporated stilbene dipeptide mimic, and 2) few attractive stabilizing forces provided by the attached short tetrapeptide strands.[32] Here we would like to emphasize that this finding does not necessarily indicate that light-triggered isomerization would not be applicable for conformational modulation of linear peptides, a suggestion strongly supported by the observed low thermodynamic stability of the folded conformation of the nonswitchable analogue **1**, and also by a very recent report by Hilvert et al.[8,33] In addition, we have shown that the light-induced conformational change, established by standard spectroscopic methods, is accompanied by a significant alteration in the translational self-diffusion coefficient of the cyclic oligopeptide. It should also be noted that all experimental and computational data are in good agreement, both with each other and with current theories. The fact that **4** and **5** but not **2** and **3** could be separated by reversed-phase HPLC may further indicate a significant conformational change upon irradiation of the cyclic peptidomimetic, resulting in considerable changes in its accessible surface and physicochemical properties.

As β-hairpins are involved in molecular recognition events in numerous vital physiological processes[34] and pathological disorders,[35] we believe that their photoswitchable mimetics should be of considerable interest in drug development,[7] and should also become widely used tools in nanotechnology.[2] Further work should address the optimization of the photochemical aspects and the incorporation of switches into specific biological systems.

Experimental Section

Synthesis: Starting materials were purchased from commercial suppliers and were used without purification. Solid-phase peptide synthesis of **4** was carried out on 2-chlorotrityl chloride resin on a 500 mg scale by use of a Fmoc/*t*Bu protection scheme. For compounds **1** and **2** solid-phase peptide synthesis was carried out on Rink amide MBHA resin on a 500 mg scale (loading rate 0.73 mmol g^{-1}) by a Fmoc/*t*Bu protection scheme. Chain elongation was performed with the Fmoc-protected amino acids (110 μmol) with PyBOP-mediated (110 μmol) coupling steps (2 h) in a mixture of diisopropylethylamine (220 μmol) and DMF (3.0 cm^3). Removal of the Fmoc groups was achieved by treatment with 20% piperidine in DMF for 5+10 min. After introduction of each amino acid, a Kaiser test[36] was performed and capping was carried out (30 min) by addition of acetic anhydride (1.5 cm^3) in dichloromethane (2.0 cm^3) and diisopropylethylamine (0.5 cm^3). The preparation of the turn mimetic part of the presented stilbene-type peptidomimetics is outlined in Scheme 3. The Fmoc-protected turn mimic was then incorporated into the peptide by standard SPPS techniques as described above. Cleavage of the products (**1** and **2**) was achieved by addition of 95% trifluoroacetic acid in dichloromethane (1 h+2×30 min), followed by filtration and concentration of the solutions under reduced pressure. In order to obtain the cyclic peptidomimetic, the linear peptide NH_2-Thr(*t*Bu)-Thr(*t*Bu)-NH-Stilbene-CO-Ile-Val-Leu-Leu-^{D}Pro-Gly-Leu-Ala-OH was cleaved from the resin with 0.5% trifluoroacetic acid in dichloromethane (4×5 cm^3), followed by filtration into a flask containing 250 μL pyridine. The combined phases were washed with H_2O and concentrated under reduced pressure. Preparative HPLC indicated that the purity of the cleaved product was approximately 95%. Cyclization of the linear peptide was achieved by HATU-mediated (110 μmol) coupling in a mixture of diisopropylethyl-



Scheme 3. Outline of the synthesis of **14**. a) $CH_2(COOH)_2$, pyridine, piperidine, 100 °C, 1.5 h, 95 %. b) CH_3OH, conc. HCl, RT, 16 h, 93 %. c) $Ni(OAc)_2$, $NaBH_4$, CH_3OH, EtOAc, H_2 (2 bar), 20 min, RT, 68 %. d) $Bu_3SnCH{=}CH_2$, $[Pd(PPh_3)_2Cl_2]$, Et_3N, DMF, 130 °C, 25 min, 78 %. e) $O[CO_2C(CH_3)_3]_2$, CH_2Cl_2, K_2CO_3 in H_2O, RT, 24 h, 70 %. f) $Pd(OAc)_2$, $(CH_3C_6H_4)_3P$, Et_3N, DMF, 120 °C, 30 min, 43 %. g) 50 % CF_3COOH in CH_2Cl_2, 15 min. h) Fmoc-Cl, dioxane, 10 % Na_2CO_3 (aq), 17 h, 82 %. i) CH_2Cl_2, conc. HCl, 22 h, 91 %.

amine (220 μmol) and DMF (3.0 cm^3) overnight. The mixture was then concentrated under reduced pressure and the *tert*-butyl protecting groups were removed with 50 % trifluoroacetic acid in dichloromethane.

Purification of the peptides was performed on a Gilson 321 HPLC system connected to a Vydac Protein & Peptide C18 (218TP) column (10 μm, 22×250 mm) with use of a gradient of acetonitrile in 0.1 % aqueous trifluoroacetic acid (10–85 % MeCN in 75 min) at a flow rate of 5 $cm^3 min^{-1}$ and with detection by UV absorbance at 230 nm (LKB 2151 absorbance detector). The fractions were further analyzed by analytical LC–MS.

Amino acid analyses were performed at the Department of Bioorganic Chemistry, Biomedical Centre, Uppsala, Sweden, on 24 h hydrolysates with an LKB 4151 alpha plus analyzer, with use of ninhydrin detection.

Experimental data: The method of preparation of the peptidomimetic **14** is outlined in Scheme 3.

3-(3′-Bromophenyl)acrylic acid (6): A mixture of 3-bromobenzaldehyde (5.00 g, 27.0 mmol), malonic acid (4.39 g, 42.2 mmol), pyridine (2.93 g, 37.1 mmol), and piperidine (7–8 drops) was heated at reflux on an oil bath (100 °C) for 1.5 h. Ice and concentrated HCl (12 cm^3) were then added and the formed crystals were filtrated and washed with HCl (1 M) and water. The product was recrystallized from 95 % ethanol and washed with cooled methanol, yielding white crystals (4.77 g, 21.0 mmol, 95 %). ^{1}H NMR (270.2 MHz, CD_3OD, 25 °C): $\delta = 6.34$ (d, $^3J(H,H) = 15.9$ Hz, 1H; CH), 7.19 (t, $^3J(H,H) = 7.8$ Hz, 1H; ArH), 7.38 (ddd, $^3J(H,H) = 1.0$, 1.8, 7.8 Hz, 1H; ArH), 7.43 (ddd, $^3J(H,H) = 1.0$, 1.8, 7.8 Hz, 1H; ArH), 7.52 (d, $^3J(H,H) = 15.9$ Hz, 1H; CH), 7.59 ppm (t, $^3J(H,H) = 1.8$ Hz, 1H; ArH); ^{13}C NMR (67.5 MHz, CD_3OD, 25 °C): $\delta = 119.5$, 122.8, 126.5, 130.2, 130.6, 132.9, 136.3, 143.4, 168.6 ppm; MS (70 eV, EI): *m*/*z* (%): 228, 226 $[M]^+$, 147, 102, 91, 75, 51.

Methyl 3-(3-bromophenyl)acrylate (7): Concentrated aqueous HCl (2–3 drops) was added to 3-(3-bromophenyl)acrylic acid (4.96 g, 21.90 mmol) in CH_3OH (15 cm^3), and the solution was stirred for 12 h. The solvent was then evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was washed with HCl (0.1 M) and water, and the water phase was re-extracted three times with dichloromethane. The combined organic phases were evaporated, yielding white crystals (4.87 g, 20.20 mmol, 93 %). ^{1}H NMR (270.2 MHz, $CDCl_3$, 25 °C): $\delta = 3.80$ (s, 3H; CH_3), 6.42 (d, $^3J(H,H) = 15.9$ Hz, 1H; CH), 7.25 (t, $^3J(H,H) = 7.9$ Hz, 1H; ArH), 7.42 (dt, $^3J(H,H) = 1.0$, 1.6, 7.9 Hz, 1H; ArH), 7.49 (ddd, $^3J(H,H) = 1.0$, 1.6, 7.9 Hz, 1H; ArH), 7.60 (d, $^3J(H,H) = 15.9$ Hz, 1H; CH), 7.65 ppm (t, $^3J(H,H) = 1.6$ Hz, 1H; ArH); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 51.8$, 119.2, 123.0, 126.6, 130.3, 130.7, 133.0, 136.4, 143.1, 166.9 ppm; MS (70 eV, EI): *m*/*z* (%): 242, 240 $[M]^+$, 184, 182, 171, 169, 104, 77, 51.

Methyl 3-(3-bromophenyl)propionate (8): $Ni(OAc)_2$ (2.46 g, 9.90 mmol) dissolved in CH_3OH (15 cm^3) and ethyl acetate (30 cm^3) was added to methyl 3-(3-bromophenyl)acrylate (1.59 g, 6.60 mmol) and $NaBH_4$ (0.75 g, 19.80 mmol) in a Parr tube. The mixture was hydrogenated (2 bar) in a Parr apparatus for 20 minutes. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2 and washed with water. The organic phase was separated, and the aqueous phase was reextracted with dichloromethane three times. The combined organic phases were filtered through Celite and $MgSO_4$ and were then concentrated under reduced pressure, yielding a yellowish oil, containing 1.5 % debrominated byproduct (1.09 g, 4.47 mmol, 68 %). ^{1}H NMR (499.9 MHz, $CDCl_3$, 25 °C): $\delta = 2.50$ (t, $^3J(H,H) = 7.6$ Hz, 2H; CH_2), 2.80 (t, $^3J(H,H) = 7.6$ Hz, 2H; CH_2), 3.55 (s, 3H; CH_3), 7.16 (dd, $^3J(H,H) = 1.5$, 7.5 Hz, 1H; ArH), 7.17 (t, $^3J(H,H) = 7.5$ Hz, 1H; ArH), 7.35 (dd, $^3J(H,H) = 1.5$, 7.5 Hz, 1H; ArH), 7.37 ppm (br t, $^3J(H,H) = 1.5$ Hz, 1H; ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 °C): $\delta = 30.7$, 35.4, 51.8, 127.2, 129.6, 128.7, 130.3, 131.6, 143.2, 172.9 ppm; MS (70 eV, EI): *m*/*z* (%): 244, 242 $[M]^+$, 184, 182, 171, 169, 104, 77, 63, 51.

2-(3-Vinylphenyl)ethylamine (9): 3-Bromophenethylamine (500.0 mg, 2.50 mmol), $[Pd(PPh_3)_2Cl_2]$ (52.6 mg, 75.0 μmol), tributylvinyltin (1.1 cm^3, 3.75 mmol), and LiCl (264.9 mg, 6.30 mmol) in DMF (1.5 cm^3) were stirred in a Smith Process Vial at 130 °C for 25 min in the microwave cavity. This procedure was repeated four times, and the combined reaction mixture was then filtered through Celite and extracted with concentrated aqueous $NaHCO_3$. The organic phase was then extracted with aqueous HCl (1 M). By addition of NaOH pellets, the pH of the aqueous solution was increased to 14, which was followed by extraction with CH_2Cl_2. The organic phase was filtered through $CaCO_3$ and concentrated under reduced pressure, yielding a yellowish oil (1.15 g, 7.81 mmol, 78 %). ^{1}H NMR (399.8 MHz, $CDCl_3$, 25 °C): $\delta = 1.01$ (br s, 2H; NH_2), 2.64 (t,

^3J(H,H) = 6.8 Hz, 2H; CH_2), 2.86 (t, ^3J(H,H) = 6.8 Hz, 2H; CH_2), 5.17 (d, ^3J(H,H) = 10.8 Hz, 1H; CH), 5.68 (d, ^3J(H,H) = 17.6 Hz, 1H; CH), 6.63 (dd, ^3J(H,H) = 10.8, 17.6 Hz, 1H; CH), 7.00 (d, ^3J(H,H) = 6.2 Hz, 1H; ArH), 7.15–7.20 ppm (m, 3H; ArH); ^{13}C NMR (100.5 MHz, $CDCl_3$, 25 °C): δ = 40.1, 43.6, 113.8, 124.1, 126.8, 128.4, 128.7, 136.9, 137.7, 140.2 ppm; MS (30 eV, ESI): *m*/*z* (%): 189, 148 $[M+H]^+$.

[2-(3-Vinylphenyl)ethyl]carbamic acid *tert*-butyl ester (10): 2-(3-Vinylphenyl)ethylamine (950.0 mg, 6.50 mmol) and di-*tert*-butyl dicarbonate (1.55 g, 7.10 mmol) were dissolved in CH_2Cl_2 (20 cm^3) and mixed with aqueous potassium carbonate (20 cm^3, 2.68 g, 19.4 mmol K_2CO_3). The heterogeneous mixture was rigorously stirred for 24 h, and the organic phase was then separated, filtered through $MgSO_4$, and concentrated. The residue was purified by column chromatography with a hexane/ethyl acetate (9:1) eluent mixture, yielding a colorless oil (113.5 mg, 4.5 mmol, 70%). ^{1}H NMR (399.8 MHz, $CDCl_3$, 25 °C): δ = 1.43 (s, 9H; CH_3^{Boc}), 2.78 (t, ^3J(H,H) = 6.9 Hz, 2H; CH_2), 3.36 (t, ^3J(H,H) = 6.9 Hz, 2H; CH_2), 4.61 (brs, 1H; NH), 5.26 (d, ^3J(H,H) = 11.0 Hz, 1H; CH), 5.74 (d, ^3J(H,H) = 17.6 Hz, 1H; CH), 6.69 (dd, ^3J(H,H) = 11.0, 17.6 Hz, 1H; CH), 7.08 (d, ^3J(H,H) = 6.0 Hz, 1H; ArH), 7.22–7.29 ppm (m, ArH3H;); ^{13}C NMR (100.5 MHz, $CDCl_3$, 25 °C): δ = 28.5 (3C), 36.2, 41.8, 79.3, 114.0, 124.4, 126.8, 128.4, 128.8, 136.8, 137.9, 139.3, 156.0 ppm; MS (30 eV, ESI): *m*/*z* (%): 495 $[2M+H]^+$ 405, 289, 248 $[M+H]^+$, 233.

Methyl *trans*-3-{3-{2-[3-(2-*tert*-butoxycarbonylaminoethyl)phenyl]vinyl}-phenyl}propionate (11): A mixture of **10** (90.0 mg, 0.3 mmol), **8** (80.2 mg, 0.33 mmol), $Pd(OAc)_2$ (3.4 mg, 15.0 μmol), tri-*o*-tolyl-phosphine (9.1 mg, 30.0 mmol), and triethylamine (0.10 cm^3, 0.90 mmol) in dimethylformamide (1.50 cm^3) was stirred in a Smith Process Vial at 120 °C for 30 min in the microwave cavity. The resulting mixture was filtered through Celite into a separation funnel. Dichloromethane (25 cm^3) was added, and the organic phase was extracted with HCl (1.0 M, 25 cm^3) and concentrated aqueous $NaHCO_3$ solution, the aqueous phase being reextracted twice. The combined organic layers were filtered through $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography with a hexane/ethyl acetate eluent mixture with a gradient of 9:1 to 1:1, yielding a white precipitate (53.1 mg, 0.13 mmol, 43%). M.p. = 80–82 °C (from EtOAc); ^{1}H NMR (399.8 MHz, $CDCl_3$, 25 °C): δ = 1.45 (s, 9H; CH_3^{Boc}), 2.66 (t, ^3J(H,H) = 7.6 Hz, 2H; CH_2), 2.69 (brt, ^3J(H,H) = 6.9 Hz, 2H; CH_2), 2.96 (t, ^3J(H,H) = 7.6 Hz, 2H; CH_2), 3.40 (brt, ^3J(H,H) = 6.9 Hz, 2H; CH_2), 3.68 (s, 3H; CH_3), 4.60 (brs, 1H; NH), 7.08 (m, 2H; CH), 7.10 (d, ^3J(H,H) = 7.3 Hz, 2H; ArH), 7.27 (dt, ^3J(H,H) = 2.2, 7.6 Hz, 2H; ArH), 7.33–7.40 ppm (m, 4H; ArH); ^{13}C NMR (100.5 MHz, $CDCl_3$, 25 °C): δ = 28.5 (3C), 31.0, 35.8, 36.3, 41.8, 51.7, 79.3, 124.6, 124.8, 126.6, 127.0, 127.7, 128.2, 128.7, 128.8, 128.9, 129.0, 137.6, 137.7, 139.5, 141.0, 156.0, 173.4 ppm; IR ($CHCl_3$): $\tilde{\nu}_{max}$ = 3446, 3055, 2984, 1725, 1433, 1262 cm^{-1}; MS (30 eV, ESI): *m*/*z* (%): 819 $[2M+H]^+$, 410 $[M+H]^+$, 354.

Methyl *trans*-3-{3-{2-[3-(2-aminoethyl)phenyl]vinyl}phenyl}propionate (12): Compound **11** (202.5 mg, 0.49 mmol) was mixed with trifluoroacetic acid in dichloromethane (50%) and stirred for 15 min. The solution was then concentrated under reduced pressure, yielding a yellowish oil (202.5 mg, 6.55 mmol, 93%). ^{1}H NMR (399.8 MHz, $CDCl_3$, 25 °C): δ = 2.72 (t, ^3J(H,H) = 7.7 Hz, 2H; CH_2), 2.96 (t, ^3J(H,H) = 7.7 Hz, 2H; CH_2), 3.02 (t, ^3J(H,H) = 7.4 Hz, 2H; CH_2), 3.41 (brt, ^3J(H,H) = 7.4 Hz, 2H; CH_2), 3.72 (s, 3H; CH_3), 6.91 (brs, 2H; NH_2), 7.06 (m, 2H; CH), 7.08 (d, ^3J(H,H) = 7.3 Hz, 2H; ArH), 7.25–7.32 (m, 4H; ArH), 7.34 (t, ^3J(H,H) = 7.7 Hz, 1H; ArH), 7.43 (d, ^3J(H,H) = 7.7 Hz, 1H; ArH), 12.10 ppm (brs, 1H; COOH); ^{13}C NMR (100.5 MHz, $CDCl_3$, 25 °C): δ = 30.9, 33.2, 35.9, 42.0, 52.7, 124.9, 126.1, 126.5, 126.8, 127.7, 127.8, 128.0, 129.1, 129.4, 129.8, 135.1, 137.4, 138.5, 140.3, 176.5 ppm; MS (30 eV, ESI): *m*/*z* (%): 620.1 (1.4) $[2M+H]^+$, 310.1 (100) $[M+H]^+$.

Methyl *trans*-3-{3-{2-{3-[2-(9*H*-fluoren-9-ylmethoxycarbonylamino)ethyl]-phenyl}vinyl}phenyl}propionate (13): Compound **12** (630.4 mg, 2.04 mmol) and 9-fluorenylmethyl chloroformate (582.3 mg, 2.25 mmol) were dissolved in a mixture of dioxane (30 cm^3) and aqueous Na_2CO_3 solution (10%), and the mixture was stirred for 17 h at room temperature. The mixture was then extracted with CH_2Cl_2, and the organic phase was separated, filtered through $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography with a hexane/ethyl acetate (2:1) eluent mixture, yielding a white solid (887.7 mg, 1.67 mmol, 82%). ^{1}H NMR (399.8 MHz, $CDCl_3$, 25 °C): δ = 2.67 (t, ^3J(H,H) = 7.7 Hz, 2H; CH_2), 2.86 (t, ^3J(H,H) = 6.7 Hz, 2H; CH_2), 2.98 (t, ^3J(H,H) = 7.7 Hz, 2H; CH_2), 3.50 (dt, ^3J(H,H) = 6.7, 6.9 Hz, 2H; CH_2), 3.69 (s, 3H; CH_3), 4.22 (t, ^3J(H,H) = 6.8 Hz, 1H; Fmoc-CH), 4.41 (d, ^3J(H,H) = 6.8 Hz, 2H; Fmoc-CH_2), 4.88 (t, ^3J(H,H) = 6.9 Hz, 1H; NH), 7.09 (m, 2H; CH), 7.11 (d, ^3J(H,H) = 7.3 Hz, 2H; ArH), 7.25–7.35 (m, 8H; ArH), 7.39 (t, ^3J(H,H) = 7.3 Hz, 2H; Fmoc-ArH), 7.58 (d, ^3J(H,H) = 7.5 Hz, 2H; Fmoc-ArH), 7.76 ppm (d, ^3J(H,H) = 7.5 Hz, 2H; Fmoc-ArH); ^{13}C NMR (100.5 MHz, $CDCl_3$, 25 °C): δ = 31.0, 35.7, 36.3, 42.3, 47.4, 51.8, 66.7, 120.1, 124.7, 124.9, 125.2, 126.6, 127.1, 127.2, 127.7, 127.8, 128.3, 128.6, 128.9, 129.0, 129.1, 137.6, 137.8, 139.3, 141.0, 141.4, 144.1, 156.4, 173.4 ppm; MS (30 eV, ESI): *m*/*z* (%): 1063.1 (22) $[2M+H]^+$, 532.1 (100) $[M+H]^+$.

***trans*-3-{3-{2-{3-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)ethyl]phenyl}vinyl}phenyl}propionic acid (14)**: Compound **17** (106.0 mg, 0.20 mmol), dissolved in dichloromethane (4.5 cm^3), was added to concentrated aqueous HCl solution (40 cm^3) and was heated at reflux at 120 °C for 22 h. The mixture was then extracted with dichloromethane, and the organic phase was separated, filtered through $MgSO_4$, and concentrated under reduced pressure, yielding a white solid (639.4 mg, 1.24 mmol, 91%). ^{1}H NMR (399.8 MHz, CD_3CN, 25 °C): δ = 2.63 (t, ^3J(H,H) = 8.0 Hz, 2H; CH_2), 2.85 (t, ^3J(H,H) = 7.0 Hz, 2H; CH_2), 2.92 (t, ^3J(H,H) = 8.0 Hz, 2H; CH_2), 3.42 (brt, ^3J(H,H) = 7.0 Hz, 2H; CH_2), 4.20 (t, ^3J(H,H) = 7.0 Hz, 1H; Fmoc-CH), 4.31 (d, ^3J(H,H) = 7.0 Hz, 2H; Fmoc-CH_2), 7.13 (d, ^3J(H,H) = 7.7 Hz, 1H; ArH), 7.16 (d, ^3J(H,H) = 7.7 Hz, 1H; ArH), 7.22 (m, 2H; CH), 7.27 (t, ^3J(H,H) = 7.7 Hz, 2H; ArH), 7.29 (t, ^3J(H,H) = 7.3 Hz, 2H; ArH), 7.38 (t, ^3J(H,H) = 7.3 Hz, 2H; ArH), 7.43 (d, ^3J(H,H) = 7.7 Hz, 2H; ArH), 7.48 (2 × brs, 2H; ArH), 7.66 (d, ^3J(H,H) = 7.3 Hz, 2H; Fmoc-ArH), 7.83 ppm (d, ^3J(H,H) = 7.3 Hz, 2H; Fmoc-ArH); ^{13}C NMR (100.5 MHz, CD_3CN and 1 drop of CD_3OD, 25 °C): δ = 30.7, 35.1, 35.9, 42.1, 47.3, 65.9, 120.0, 124.4, 124.5, 125.3, 126.6, 127.0, 127.1, 127.6, 127.7, 127.72, 128.2, 128.6, 128.63, 128.8, 137.6, 137.7, 140.0, 141.3, 141.6, 144.4, 156.4, 173.2 ppm; MS (30 eV, ESI): *m*/*z* (%): 1035.0 (14) $[2M+H]^+$, 518.1 (1) $[M+H]^+$.

$(C_2H_5)_2$N-$(CH_2)_2$CO-Leu-Ala-Thr-Thr-^{D}Pro-Gly-Ile-Val-Leu-Leu-NH_2 (1): 84.4 mg, 75 μmol, 20.6%; $[\alpha]_D$ = −73.5° (methanol, 17 °C, pH 3.2); MS (ESI, 30 eV) *m*/*z* (%): 1123.4 (11) $[M+H]^+$, 562.6 (100) $[M+2H]^+$; $D = 0.90 \times 10^{-6}$ $cm^2 s^{-1}$ ([D_6]DMSO, 25 °C); amino acid analysis: Thr 2.01, Pro 1.03, Gly 1.03, Ala 1.01, Val 0.93, Ile 0.93, Leu 3.05 (71% peptide).

***trans*-$(C_2H_5)_2$N-$(CH_2)_2$CO-Leu-Ala-Thr-Thr-$(CH_2)_2$PhCH=CHPh$(CH_2)_2$-Ile-Val-Leu-Leu-NH_2 (2)**: 29.9 mg, 24 μmol, 6.6%; $[\alpha]_D$ = −64.3° (methanol, 19 °C, pH 3.0); $D = 0.7 \times 10^{-6}$ $cm^2 s^{-1}$ ([D_6]DMSO, 25 °C); MS (ESI, 30 eV) *m*/*z* (%): 1246.7 (17) $[M+H]^+$, 624.5 (100) $[M+2H]^+$; amino acid analysis: Thr 2.08, Ala 1.00, Val 0.90, Ile 0.89, Leu 3.00 (71% peptide).

***cis*-$(C_2H_5)_2$N-$(CH_2)_2$CO-Leu-Ala-Thr-Thr-$(CH_2)_2$PhCH=CHPh$(CH_2)_2$-Ile-Val-Leu-Leu-NH_2 (3)**: Quantum yield = 2.9%; MS (ESI, 30 eV) *m*/*z* (%): 1246.7 (0.5) $[M+H]^+$, 624.5 (100) $[M+2H]^+$; $D = 0.7 \times 10^{-6}$ $cm^2 s^{-1}$ ([D_6]DMSO, 25 °C).

***trans*-Cyclo(-^{D}Pro-Gly-Leu-Ala-Thr-Thr-$(CH_2)_2$PhCH=CHPh$(CH_2)_2$-Ile-Val-Leu-Leu-) (4)**: $[\alpha]_D^{19}$ = −42.9 (methanol); MS (30 eV, ESI): *m*/*z* (%): 1256.4 (17) $[M+H]^+$, 629.1 (100) $[M+2H]^{2+}$; $D = 1.4 \times 10^{-6}$ $cm^2 s^{-1}$ ([D_6]DMSO, 25 °C); amino acid analysis: Thr 2.01, Pro 1.06, Gly 1.03, Ala 0.96, Val 0.99, Ile 0.94, Leu 3.01 (54% peptide).

***cis*-Cyclo(-^{D}Pro-Gly-Leu-Ala-Thr-Thr-$(CH_2)_2$PhCH=CHPh$(CH_2)_2$-Ile-Val-Leu-Leu-) (5)**: Quantum yield = 1.2% (300 nm); $[\alpha]_D^{19}$ = −66.8 (methanol); MS (30 eV, ESI): *m*/*z* (%): 1256.4 (17) $[M+H]^+$, 629.1 (100) $[M+2H]^+$; $D = 1.8 \times 10^{-6}$ $cm^2 s^{-1}$ ([D_6]DMSO, 25 °C).

Photoisomerization: Photochemical reactions were performed in dimethyl sulfoxide under N_2 gas flow with use of an Oriel 1000 W Xe ARC light source and a 300 nm or a 280 nm Oriel UV filter. The emitted light intensity was determined at the wavelengths of isomerizations (280 or 300 nm) with use of a UV enhanced Silica photodiode (5.8 mm^2) attached to a current meter.

NMR measurements: NMR spectra were recorded on Varian INOVA (^{1}H at 499.9 MHz), Jeol EX-400 (^{1}H at 399.8, and ^{13}C at 100.5 MHz), or Jeol EX-270 (^{1}H at 270.2, and ^{13}C at 67.5 MHz) spectrometers. Signal as-

signment was carried out by use of COSY,[37] NOESY,[38] ROESY,[39] wettntocsy,[40] and wetroesy[40] experiments performed at 25 °C. NOE effects were measured with mixing times of between 0.3 and 1.0 s. The pH values of the NMR samples were in the 3.2–3.5 range for CH_3OH/CD_3OD and the 4.6–4.8 range for [D_6]DMSO solutions. These values were measured with an NMR electrode and are uncorrected. Amide proton temperature coefficients $\Delta\delta_{NH}/\Delta T$ (ppb K^{-1}) were measured for 3 mmol dm^{-3} samples in DMSO (298–388 K), and CH_3OH/CD_3OD (1:1, 188–328 K) solutions. For the PGSE experiments performed in [D_6]DMSO at 25 °C, *z*-gradients were employed and 16 scans were acquired. 1 s relaxation delay, 9 ms gradient pulse duration, 20 ms diffusion delay, 5 ms storage delay was used and the gradient pulse strength was arrayed between 0 and 20 gauss cm^{-1} (20 steps). The diffusion coefficients were calculated from the known coefficient of H_2O in [D_6]DMSO (9×10^{-6} $cm^2 s^{-1}$).[28]

CD spectroscopy: Optical rotation was measured with a Perkin–Elmer 241 polarimeter and samples in methanol. Circular dichroism spectra were obtained on a JASCO J-810 spectropolarimeter from 190 to 400 nm in a 0.2 mm pathlength cell. Five scans were accumulated at ambient temperature with a scanning speed of 100 nm min^{-1}, with sample solutions (0.56 mmol dm^{-3}) in methanol (pH 4.6–4.8). UV spectra were measured with a Varian Cary 3 spectrometer.

Mass spectrometry: ESI-mass spectra (ESI = electrospray ionization) were obtained with a Finnigan ThermoQuest AQA mass spectrometer (ESI 30 eV, probe temperature 100 °C) fitted with a Gilson 322-H2 Gradient Pump system and a SB-C18 column. A water/acetonitrile/formic acid (0.05 %) mobile phase was used with a gradient of 20 to 100 % acetonitrile during 3–5 min.

Computational chemistry: Theoretical conformational analysis without experimental constraints was performed first for observation of folding tendencies. In addition, calculations for description of the solution structures were carried out with constraints derived from ROESY/NOESY cross peaks. The NMR structures obtained for solutions in methanol were hence visualized by use of the program Macromodel 7.0.[41] The OPLS-AA all-atom force field and the General Born/Solvent Accessible (GB/SA) surface area method developed by Still[42] were used. The number of torsion angles allowed to vary during each Monte Carlo step ranged from 1 to $n-1$, where n equals the total number of rotatable bonds. Amide bonds were fixed in *trans* configurations. Structural constraints derived from NOESY cross peaks (illustrated in Scheme 2) were introduced by use of the DISC command (1.0–5.0 Å) as implemented in Macromodel 7.0. Conformational searches were conducted by use of the Systematic Unbound Multiple Minimum (SUMM) search method[43] implemented in the Batchmin program. 10000 Monte Carlo step runs were performed and those conformations within 25 kJ mol^{-1} of the global minimum were kept. PR Conjugate Gradient minimization with 5000 iterations was used in the conformational search. The threonine hydroxy groups were protected with methyl groups during the computation in order to compensate for Macromodel's well-known overestimation of electrostatic forces. The structures of lowest energy are depicted in Figure 1.

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