**Supplementary Material**

**A photochromic azobenzene peptidomimetic of a β-turn model peptide structure as a conformational switch**

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Peptide1 HPLC-TIC.TIF

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**Figure S1.** RP-HPLC: tr =3.17 min (*cis* isomer) and 3.78 min (*trans* isomer), gradient 35% to 55% of B in 5 min; Mr = calcd. for C116 H170 N29 O25 S1: 2402,88 ESI-MS: m/z: 1202,47 [M+2H]2+; 802,02 [M+3H]3+

RP-HPLC system is an Alliance Chromatography (Waters, Milford Massachusetts, USA) with a Bioshell A160 C18 (Sigma Aldrich, Milano Italy; 1.7 μm 2.1× 50 mm) column at 35°C, at 0.6 mL/min coupled to a single quadrupole ESI-MS Micromass ZQ (Waters, Milford Massachusetts, USA).



**Figure S2.** UV-Vis absorption spectra at several irradiation time for (a) azobenzene **3**, (b) aminoazobenzene AMPB **2**. and (c) azopeptide **1**, exciting at 440 nm.



**Figure S3.** Superimposed TOCSY spectra of azopeptide **1** in TFE:H2O 1:1 (30°C) after 1 hour irradiation (red, 53% *cis* and 47% *trans* form) and 2 weeks relaxation (blue, 86% *trans* and 14% *cis* form). Assignments are described using one letter amino acid code. X refers to the azobenzene moiety (Azb) (see Table S1). Amino acids 1 to 20 refer to the *trans* form and 101 to 120 to the *cis* form.



**Figure S4.** NOESY spectrum (400 ms mixing time) of azopeptide **1** in TFE:D2O 1:1 (30°C) after 1 hour irradiation (53% *cis* and 47% *trans* form). Assignments are described using one letter amino acid code. X refers to the azobenzene moiety (Azb) (see Table S1). Amino acids 1 to 20 refer to the *trans* form and 101 to 120 to the *cis* form.

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| **Table S1. 1H NMR assignment of the *trans* form of azopeptide 1 in TFE:H2O 1:1 (30 °C)**a | | | | |
| Residue | HN | H | H | Other side chain H |
| Thr1 |  | 4.13 | 3.91 | H 1.32 |
| Pro2 | - | 4.46 | 2.26, 1.89 | H 2.01, 1.96 H 3.71, 3.66 |
| Arg3 | 8.05 | 4.41 | 1.89, 1.78 | H 1.64 H 3.19 |
| Val4 | 7.86 | 4.00 | 2.01 | H 0.89, 0.88 |
| Glu5 | 8.57 | 4.33 | 2.05, 1.94 | H 2.27 |
| Arg6 | 8.05 | 4.39 | 1.95, 1.80 |  |
| Azb7b | 8.21 | 4.46 | - | H*o* 7.42 H*m* 7.78 |
| Azb8b | - | 3.77 | - | H*o* 7.45 H*m* 7.80 |
| Thr9 | 7.61 | 4.30 | 4.18 | H 1.16 |
| Val10 | 7.46 | 3.94 | 1.88 | H 0.72, 0.71 |
| Phe11 | 7.36 | 4.57 | 3.04, 2.87 | H 7.09 H 7.24 H 7.18 |
| Leu12 | 7.38 | 4.34 | 1.48, 1.40 | H 0.79 |
| Ala13 | 7.60 | 4.38 | 1.24 |  |
| Pro14 | - | 4.19 | 2.07, 1.57 | H 1.79 H 3.57, 3.33 |
| Tyr15 | 7.22 | 4.34 | 2.92, 2.79 | H 6.99 H 6.82 |
| Gly16 | 8.02 | 3.93, 3.74 |  |
| Trp17 | 7.49 | 4.62 | 3.33, 3.23 | H 7.20 H 9.70 H 7.51 H 7.39 H 7.09 H 7.17 |
| Met18 | 7.70 | 4.31 | 1.92, 1.81 | H 2.12 |
| Val19 | 7.47 | 4.10 | 2.08 | H 0.89 |
| Lys20 | 7.56 | 4.23 | 1.84, 1.74 | H 1.39 H 1.68 H 2.97 |
| a 1H chemical shifts are referenced to the residual protiated solvent signal ( 1H 3.88 ppm).  b Azb7 and Azb8 are defined as moieties linked to N=N group: H protons correspond to CH2 groups; aromatic *ortho* (H*o*) and *meta* (H*m*) positions are named with respect to the CH2 substituent. | | | | |

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| **Table S2. 1H NMR assignment of the *cis* form of azopeptide 1 in TFE:H2O 1:1 (30 °C).**a Only residues showing 1H chemical shift differences with the *trans* form are reported. | | | | |
| Residue | HN | H | H | Other side chain H |
| Val4 | 7.88 | 4.07 | 2.02 | H 0.90, 0.89 |
| Glu5 | 8.47 | 4.33 | 2.02, 1.90 | H 2.23 |
| Arg6 | 8.04 | 4.34 | 1.89, 1.76 | H 1.62 H 3.15 |
| Azb7b | 8.12 | 4.30, 4.27 | - | H*o* 7.15 H*m* 6.82 |
| Azb8b | - | 3.63 | - | H*o* 7.21 H*m* 6.83 |
| Thr9 | 7.67 | 4.22 | 4.15 | H 1.11 |
| Val10 | 7.43 | 4.01 | 1.90 | H 0.70, 0.69 |
| Phe11 | 7.57 | 4.63 | 3.11, 2.87 | H 7.10 H 7.24 H 7.17 |
| Leu12 | 7.51 | 4.37 | 1.52 | H 0.80 |
| Ala13 | 7.67 | 4.43 | 1.24 |  |
| Pro14 | - | 4.21 | 2.10, 1.61 | H 1.85 H 3.61, 3.39 |
| Tyr15 | 7.20 | 4.35 | 2.92, 2.81 | H 6.99 H 6.82 |
| Trp17 | 7.49 | 4.65 | 3.33, 3.23 |  |
| Met18 | 7.74 | 4.33 | 1.92, 1.81 | H 2.15 |
| Val19 | 7.50 | 4.12 | 2.08 | H 0.91 |
| a 1H chemical shifts are referenced to the residual protiated solvent signal  ( 1H 3.88 ppm)  b Azb7 and Azb8 are defined as moieties linked to N=N group: H protons correspond to CH2 groups; aromatic *ortho* (H*o*) and *meta* (H*m*) positions are named with respect to the CH2 substituent. | | | | |

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| **Table S3. 1H NMR assignment of the *trans* form of azopeptide 1 in ACN:H2O 1:1 (25 °C)**a | | | | |
| Residue | HN | H | H | Other side chain H |
| Thr1 |  | 3.93 | 3.75 | H 1.17 |
| Pro2 | - | 4.33 | 2.16, 1.77 | H 1.88, 1.85 H 3.64, 3.56 |
| Arg3 | 8.01 | 4.24 | 1.74, 1.61 | H 1.49 H 3.06 |
| Val4 | 7.86 | 3.92 | 1.90 | H 0.78, 0.77 |
| Glu5 | 8.41 | 4.18 | 1.93, 1.79 | H 2.13 |
| Arg6 | 7.98 | 4.25 | 1.81, 1.66 | H 1.50 H 3.07 |
| Azb7b | 8.21 | 4.37 | - | H*o* 7.39 H*m* 7.74 |
| Azb8b | - | 3.71 | - | H*o* 7.45 H*m* 7.76 |
| Thr9 | 7.84 | 4.19 | 4.10 | H 1.05 |
| Val10 | 7.52 | 3.95 | 1.81 | H 0.61, 0.61 |
| Phe11 | 7.61 | 4.47 | 3.00, 2.73 | H 7.07 H 7.17 H 7.14 |
| Leu12 | 7.52 | 4.19 | 1.40 | H 0.70, 0.69 |
| Ala13 | 7.64 | 4.36 | 1.13 |  |
| Pro14 | - | 4.13 | 1.98, 1.50 | H 1.74, 1.72 H 3.50, 3.35 |
| Tyr15 | 7.39 | 4.29 | 2.89, 2.72 | H 6.95 H 6.69 |
| Gly16 | 7.82 | 3.82, 3.60 |  |
| Trp17 | 7.47 | 4.50 | 3.13 | H 7.10 H 9.90 H 7.44 H 7.31 H 6.98 H 7.05 |
| Met18 | 7.67 | 4.18 | 1.84, 1.70 | H 2.11 H 1.92 |
| Val19 | 7.50 | 4.04 | 1.96 | H 0.79, 0.78 |
| Lys20 | 7.53 | 4.06 | 1.68, 1.58 | H 1.25 H 1.52 H 2.83 |
| a 1H chemical shifts are referenced to the residual protiated solvent signal ( 1H 1.94 ppm)  b Azb7 and Azb8 are defined as moieties linked to N=N group: H protons correspond to CH2 groups; aromatic *ortho* (H*o*) and *meta* (H*m*) positions are named with respect to the CH2 substituent. | | | | |

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| **Table S4. 1H NMR assignment of the *cis* form of azopeptide 1 in ACN:H2O 1:1 (25 °C).**a Only residues showing 1H chemical shift differences with the *trans* form are reported. | | | | |
| Residue | HN | H | H | Other side chain H |
| Val4 | 7.87 | 3.95 | 1.90 | H 0.79, 0.77 |
| Glu5 | 8.35 | 4.18 | 1.90, 1.75 | H 2.09 |
| Arg6 | 7.97 | 4.19 | 1.74, 1.60 | H 1.47 H 3.04 |
| Azb7b | 8.13 | 4.21 | - | H*o* 7.10 H*m* 6.76 |
| Azb8b | - | 3.55 | - | H*o* 7.17 H*m* 6.77 |
| Thr9 | 7.81 | 4.16 | 4.05 | H 0.98 |
| Val10 | 7.54 | 3.98 | 1.84 | H 0.63, 0.61 |
| Phe11 | 7.72 | 4.50 | 3.01, 2.75 | H 7.07 |
| Leu12 | 7.58 | 4.20 | 1.41 | H 0.69 |
| Ala13 | 7.68 | 4.37 | 1.14 |  |
| a 1H chemical shifts are referenced to the residual protiated solvent signal ( 1H 1.94 ppm)  b Azb7 and Azb8 are defined as moieties linked to N=N group: H protons correspond to CH2 groups; aromatic *ortho* (H*o*) and *meta* (H*m*) positions are named with respect to the CH2 substituent. | | | | |