Supporting Information for

On the Transition from a Biomimetic Molecular Switch to a Rotary Molecular Motor

Marco Paolino,[a,*] Tommaso Giovannini,[b] Madushanka Manathunga,[c] Loredana Latterini,[d] Giulia Zampini,[d] Robin Pierron,[e] Jérémie Léonard,[e] Stefania Fusi,[a] Gianluca Giorgi,[a] Germano Giuliani,[a] Andrea Cappelli,[a] Chiara Cappelli,[b] Massimo Olivucci[a,c,*]

[a] Dipartimento di Biotecnologie, Chimica e Farmacia, Università di Siena, Via A. Moro 2, 53100 Siena, Italy;

[b] Scuola Normale Superiore, Piazza dei Cavalieri 7, 56126 Pisa, Italy.

[c] Chemistry Department, Bowling Green State University, USA;

[d] Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia, Via Elce di Sotto, 8, 06123 Perugia, Italy;

[e] Université de Strasbourg, CNRS, Institut de Physique et Chimie des Matériaux de Strasbourg, UMR 7504, F-67000 Strasbourg, France ;

E-mail: paomar@oneonline.it, molivuc@bgsu.edu

Materials and Methods

Synthesis.

All chemicals used were of reagent grade. Yields refer to purified products and are not optimized. Merck silica gel 60 (230−400 mesh) was used for column chromatography. Merck TLC plates and silica gel 60 F_{254} were used for TLC. ^{1}H NMR spectra were recorded at 400 MHz and the ^{13}C NMR were recorded at 100 MHz using a Bruker DRX-400 AVANCE spectrometer in the indicated solvents (TMS as internal standard). The values of the chemical shifts are expressed in ppm and the coupling constants (*J*) in Hz. An Agilent 1100 LC/MSD operating with an electrospray source was used in mass spectrometry experiments. The absorption spectra were recorded with a PerkinElmer Lambda 40 in the indicated solvent.

Procedure for the synthesis of the compounds **5**, *E*-**6**, *and Z*-**6**.

To a solution of N-Boc-2-pyrrolidinone (0.50 g, 2.70 mmol) dissolved in anhydrous THF (10 mL), a 1 M solution of lithium hexamethyldisilazide (LiHMDS) in anhydrous THF (3.25 mL, 3.25 mmol) was added at −78 °C under a nitrogen atmosphere. After 1 h, a solution of compound **4**[1] (0.79 g, 4.48 mmol) and $BF_3{\cdot}Et_2O$ (4.48 mmol, 565 μL) in anhydrous THF (5 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h. Then, NH_4Cl (s.s.) was added, and the crude was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The oily residue was dissolved in CH_2Cl_2 (10 mL), and trifluoroacetic acid (1.0 mL) was added. The resulting reaction mixture was stirred at room temperature for 30 min. Then, $NaHCO_3$ (s.s.) was added and the crude was extracted with CH_2Cl_2. The residue was purified by flash chromatography on silica gel (1:1, ethyl acetate/petroleum ether) to obtain compound **5** (0.31 g, yield 47%) and **6** (*E*/*Z* ratio = 8:2, 0.31 g, yield 47%).

3-(6-methoxy-2-methyl-1H-inden-3-yl)pyrrolidin-2-one (**5**).

Title compound was obtained as a pale yellow solids (0.31 g, yield 47%). An analytical sample of **5** was obtained by recrystallization from ethyl acetate by slow evaporation. ^{1}H NMR ($CDCl_3$, 400 MHz): 2.07

(s, 3H), 2.27-2.43 (m, 2H), 3.29 (m, 2H), 3.40 (m, 1H), 3.45-3.56 (m, 2H), 3.79 (s, 3H), 6.43 (br s, 1H), 6.75 (dd, *J* = 8.2, 2.3, 1H), 6.97 (d, *J* = 1.9, 1H), 7.07 (d, *J* = 8.3, 1H). MS(ESI): *m/z* 266.1 ($M + Na^+$).

(E)-3-(5-methoxy-2-methyl-2,3-dihydro-1H-inden-1-ylidene)pyrrolidin-2-one (*E*-**6**).[1]

An analytical sample of isolated *E*-**6** was obtained by flash chromatography on silica gel using petroleum ether/ethyl acetate (6:4) as eluent and subsequent crystallization from ethyl acetate by slow evaporation of the solvent. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.18 (d, *J* = 6.9, 3H), 2.57 (d, *J* = 16.5, 1H), 3.06 (m, 1H), 3.13-3.29 (m, 2H), 3.39-3.63 (m, 2H), 3.83 (s, 3H), 4.21 (m, 1H), 5.61 (br s, 1H), 6.83 (dd, *J* = 8.6, 2.2, 1H), 6.88 (s, 1H), 7.40 (d, *J* = 8.6, 1H). MS(ESI): *m/z* 266.1 ($M + Na^+$).

(Z)-3-(5-methoxy-2-methyl-2,3-dihydro-1H-inden-1-ylidene)pyrrolidin-2-one (*Z*-**6**).

An analytical sample of isolated *Z*-**6** was obtained by flash chromatography on silica gel using petroleum ether/ethyl acetate (6:4) as the eluent and subsequent crystallization from ethyl acetate by slow evaporation of the solvent. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.10 (d, *J* = 7.0, 3H), 2.49 (d, *J* = 16.1, 1H), 2.82-3.10 (m, 3H), 3.19 (dd, *J* = 16.1, 7.2, 1H), 3.47 (t, *J* = 6.7, 2H), 3.81 (s, 3H), 5.95 (br s, 1H), 6.75 (s, 1H), 6.79 (d, *J* = 8.8, 1H), 9.04 (d, *J* = 8.8, 1H). MS(ESI): *m/z* 266.1 ($M + Na^+$).

3-(5-hydroxy-2-methyl-2,3-dihydro-1H-inden-1-ylidene)pyrrolidin-2-one (**2**).

The synthesis of compound **2** was conducted separately on both precoursor compounds **5** and **6** reported here as method A and B.

Method A: To a solution of compound **5** (0.10 g, 0.41 mmol, *E*/*Z* mixture 8:2) in dichloromethane (10 mL) cooled to 0 °C was added dropwise a solution (1 M in CH_2Cl_2) of BBr_3 (4.1 mL, 4.1 mmol). The resulting mixture was stirred for 5 h at room temperature. Then, a saturated $NaHCO_3$ solution was added until the gas evolution ceased. The reaction mixture was extracted with ethyl acetate, and the organic layer was dried over sodium sulphate and evaporated under reduced pressure. Purification of residue by flash chromatography with petroleum ether-ethyl acetate (1:1) as the eluent gave pure compound **2** (0.09 g, yield 96%) as a pale yellow solid (9:1 mixture of *E*/*Z* isomers).

Method B: To a solution of compound **6** (0.20 g, 0.82 mmol, *E*/*Z* mixture 8:2) in dichloromethane (20 mL) cooled to 0 °C was added dropwise a solution (1 M in CH_2Cl_2) of BBr_3 (8.2 mL, 8.2 mmol). The resulting mixture was stirred for 5 h at room temperature. Then, a saturated $NaHCO_3$ solution was added until the gas evolution ceased. The reaction mixture was extracted with ethyl acetate, and the organic layer was dried over sodium sulphate and evaporated under reduced pressure. Purification of residue by flash chromatography with petroleum ether-ethyl acetate (1:1) as the eluent gave pure compound **2** (0.18 g, yield 96%) as a pale yellow solid (9:1 mixture of *E*/*Z* isomers).

(E)-3-(5-hydroxy-2-methyl-2,3-dihydro-1H-inden-1-ylidene)pyrrolidin-2-one (*E*-**2**).

An analytical sample of isolated *E*-**2** was obtained by flash chromatography on silica gel using petroleum ether/ethyl acetate (7:3). ^{1}H NMR (CD_3OD, 400 MHz): 1.09 (d, *J* = 6.9, 3H), 2.48 (d, *J* = 16.4, 1H), 2.90-3.02 (m, 3H), 3.37-3.54 (m, 2H), 4.09 (m, 1H), 6.72 (dd, *J* = 8.4, 2.0, 1H), 6.76 (s, 1H), 7.36 (d, *J* = 8.5, 1H). ^{13}C NMR (CD_3OD, 100 MHz): 23.4, 28.4, 37.5, 40.4, 40.6, 113.2, 115.4, 118.8, 128.1, 132.5, 151.6, 155.4, 160.4, 175.1. MS(ESI): m/z 252.3 ($M + Na^+$).

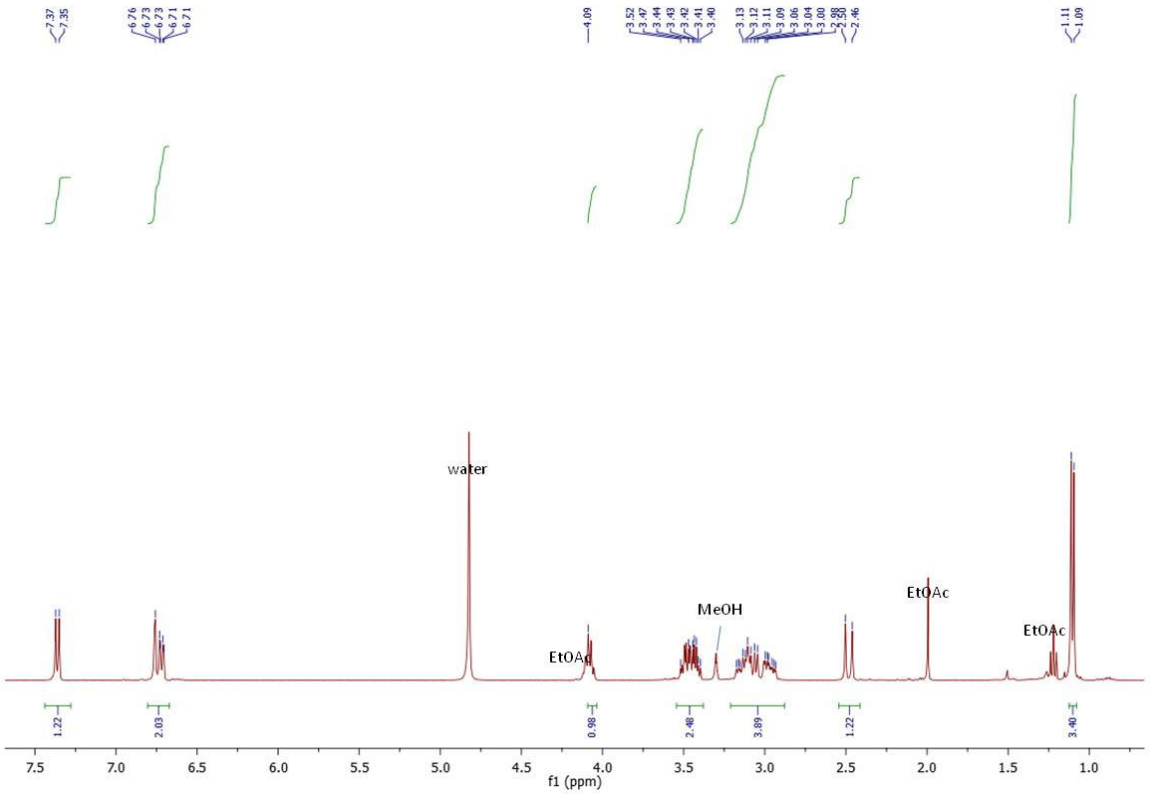

Figure SI-1. ^{1}H NMR of compound *E*-**2** in methanol-$_{d4}$.

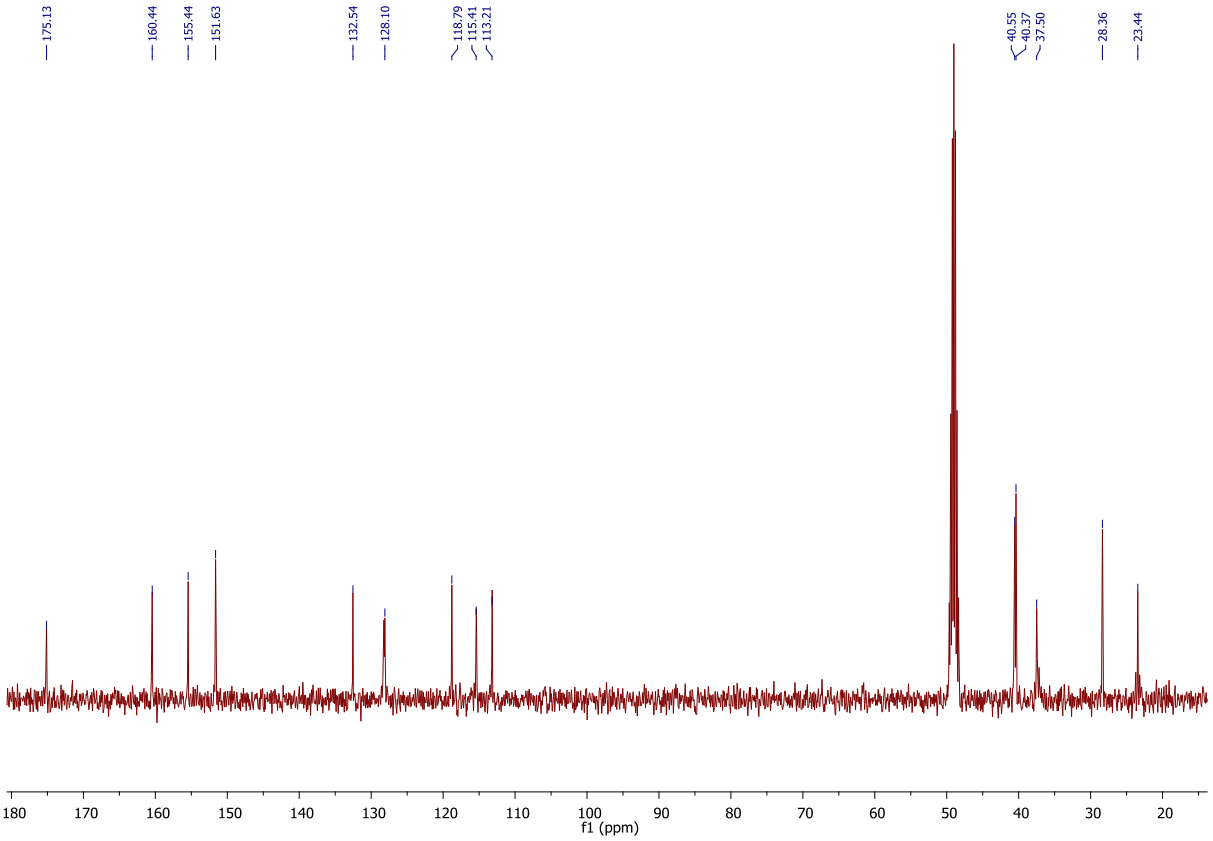

Figure SI-2. ^{13}C NMR of compound *E*-**2** in methanol-$_{d4}$.

(Z)-3-(5-hydroxy-2-methyl-2,3-dihydro-1H-inden-1-ylidene)pyrrolidin-2-one (*Z*-**2**).

After photochemical reaction ^{1}H NMR data of the geometric isomer *Z*-**2** are derived (see Fig. SI-5, line B). ^{1}H NMR (CD_3OD, 400 MHz): 1.02 (d, *J* = 6.8, 3H), 2.39 (d, *J* = 15.8, 1H), 2.83-3.18 (m, 4H), 3.34-3.43 (m, 2H), 6.54 (dd, *J* = 8.6, 2.3, 1H), 6.59 (s, 1H), 8.73 (d, *J* = 8.7, 1H).

X-Ray crystallography.

Single crystals of **5**, *E*-**6** and *Z*-**6** were submitted to X-ray data collection on an Oxford-Diffraction Xcalibur Sapphire 3 diffractometer with a graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) at 293 K. The structures were solved by direct methods implemented in SHELXS-97 program.[2] The refinements were carried out by full-matrix anisotropic least squares on F^2 for all reflections for non-H atoms by means of the SHELXL program.[3] Crystallographic data for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no.

CCDC 2062739 (**5**), CCDC 2062740 (*E*-**6**) and CCDC 2062741 (*Z*-**6**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; (fax: +44 (0) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk).

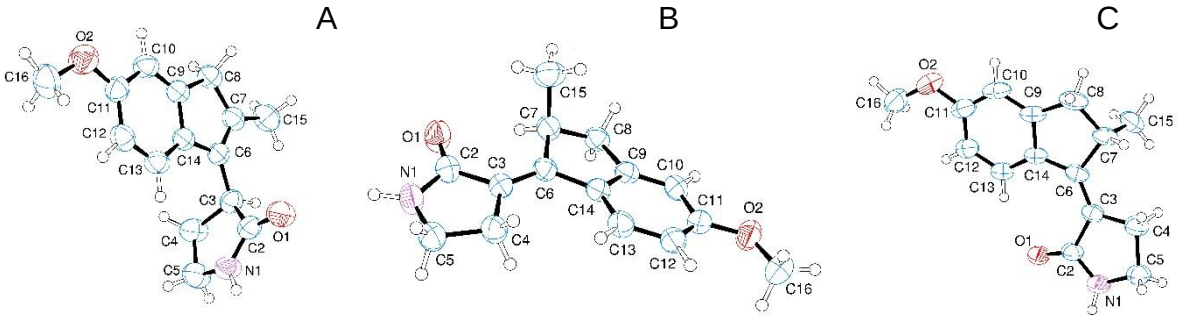

Figure SI-3. Crystallographic structures of racemic compounds **5** (A), *E*-**6** (B) and *Z*-**6** (C). Ellipsoids enclose 50% probability.

Photoisomerization Quantum Yields.

Absorption spectra were recorded on a PerkinElmer - Lambda 800 spectrophotometer. The sample was irradiated using a xenon lamp equipped with a monochromator for the selection of the excitation wavelength (315 or 350 nm). A potassium ferrioxalate solution was used as an actinometer to determine the light source intensity at the different excitation wavelengths. The chromatographic analysis was performed using a Waters apparatus equipped with a Lux cellulose 1 column and a UV−vis diode array detector. The protocols followed for the quantum yield measurement of the neutral and anionic forms of *E*-**2** are also reported in refs [4] and [5], respectively.

Elettronic Circular Dichroism.

The ECD spectra were recorded by use of a Jasco model J-810 spectropolarimeter at 25 °C using rectangular quartz cell with a 0.1 cm path length. UV CD spectra were acquired within 180-400 nm range, with a resolution of 0.5 nm.

Simulation

Generation of initial conditions. In order to generate an ensemble of initial conditions for each molecule, the following procedure was used. Each molecular structure was constructed and optimized at MP2/6-31G*/PCM level. The optimized structures were embedded in cubic solvent boxes of size 70x70x70 Å3. The solute-solvent systems were then used to carry out 5 ns long (0.3 ns annihilation, 0.7 ns equilibration and 4 ns production) MD simulations at room temperature. This was done using NPT ensemble with periodic boundary conditions. The simulations were performed using GROMACS molecular dynamics package[6] with OPLS parameters[7] to describe solute and solvent molecules. From the production MD section, 400 geometry snapshots that separates from each other by 100 ps and corresponding velocities were extracted. A solute-solvent shell containing all solvent molecules located within 20 Å distance of any solute atom was selected from the extracted geometry snapshots. At this stage, a quantum mechanics/molecular mechanics (QM/MM) setup was defined for solute-solvent systems (Fig. SI-4).

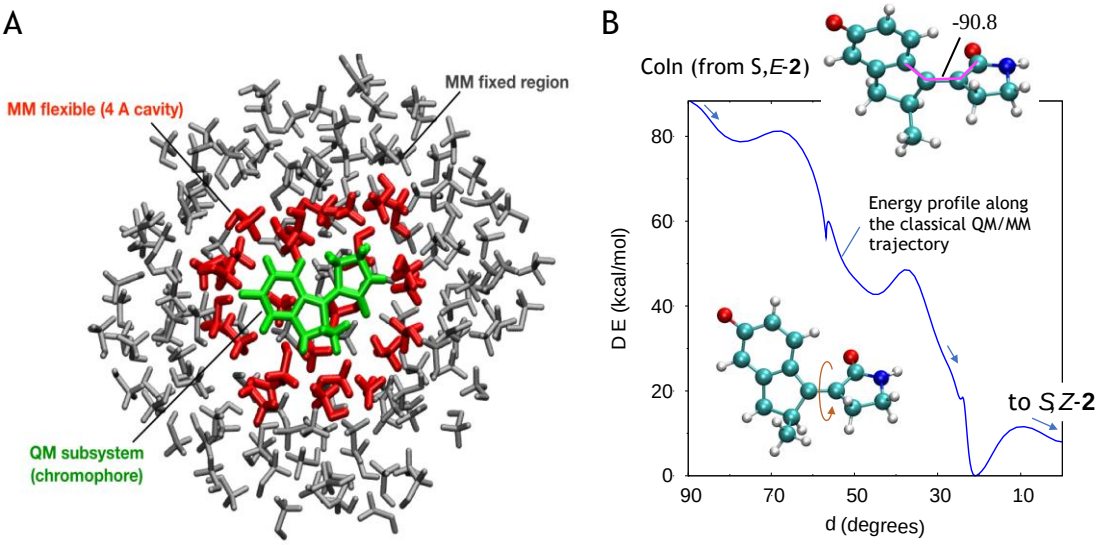

Figure SI-4. The QM/MM setup used to compute the ground state equilibrium distribution and the ground state relaxation trajectory. A. The chromophore (green) is treated at QM level whereas the rest at MM level. A solvent shell located within 4 Å of any chromophore atom (red) is allowed to move at MM level during the dynamics. The remaining solvent molecules (grey) are kept frozen. B. Energy decrease along the classical QM/MM trajectory released from the optimized *E*-**2** conical intersection (CoIn) necessary to model the ECD spectral progression.

Starting from each solute-solvent shell, 200 fs long ground state QM/MM trajectories were propagated at room temperature. For this purpose, a CASSCF/6-31G*/OPLS protocol was used. An active space comprising of 12 electrons in 11 orbitals was employed in the QM section. All QM/MM calculations were performed using Molcas/Tinker computer packages.[8,9] The final geometries and velocities from above ground state trajectories are the initial conditions required to simulate the stationary ECD spectra at room temperature.

QM/Fluctuating Charge - QM/FQ. All the QM/FQ calculations were performed using a locally modified version of the Gaussian 16 suite of programs.[10] The QM part is treated at the CAM-B3LYP/6-311+G* level of theory. The FQ parameters, i.e. atomic electronegativity (χ) and chemical hardness (η), exploited to model the methanol solution are: C ($\chi = 0.18$, $\eta = 0.17$), O ($\chi = 0.37$, $\eta = 0.95$), H ($\chi = 0.00$, $\eta = 0.52$).

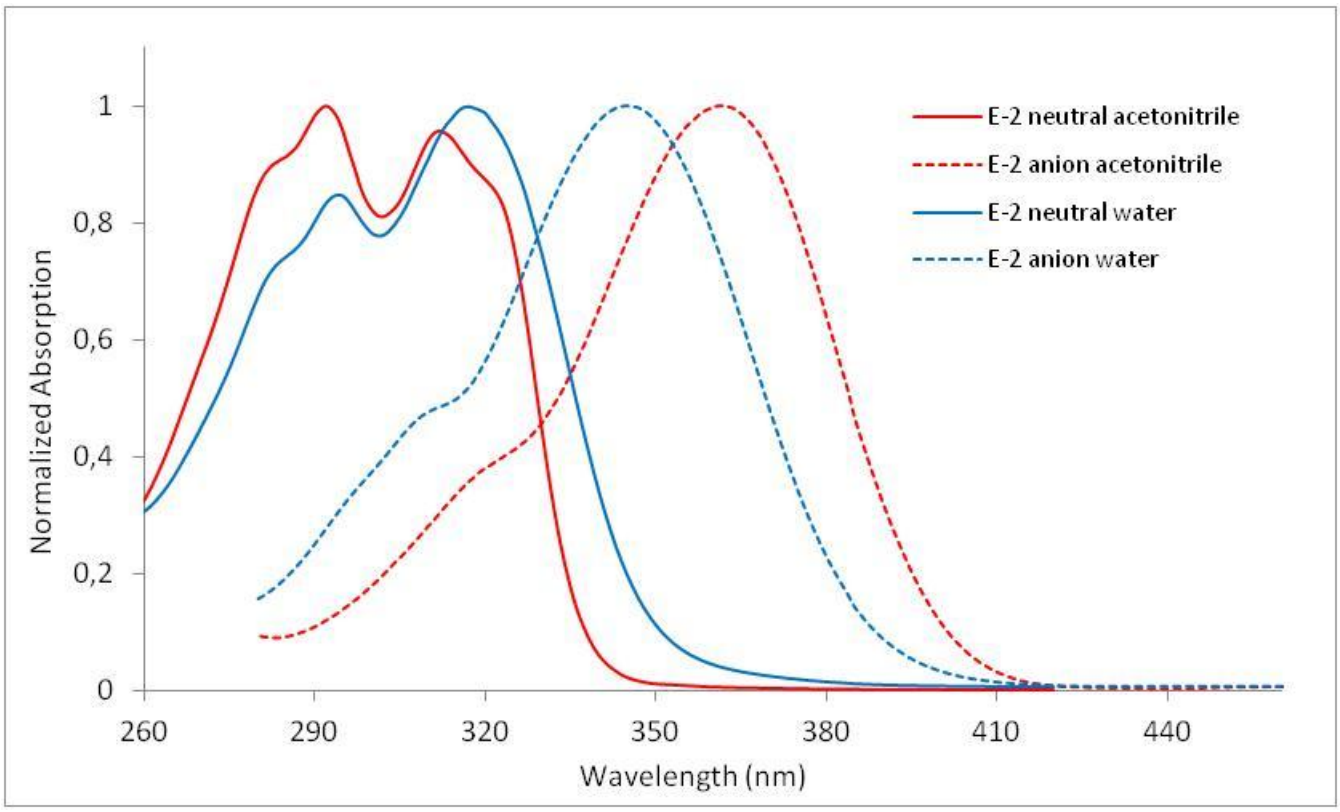

Figure SI-5. Normalized absorption spectra of the neutral (solid lines) and anionic (dashed lines) forms of *E*-**2** in acetonitrile (red) and water (blue).

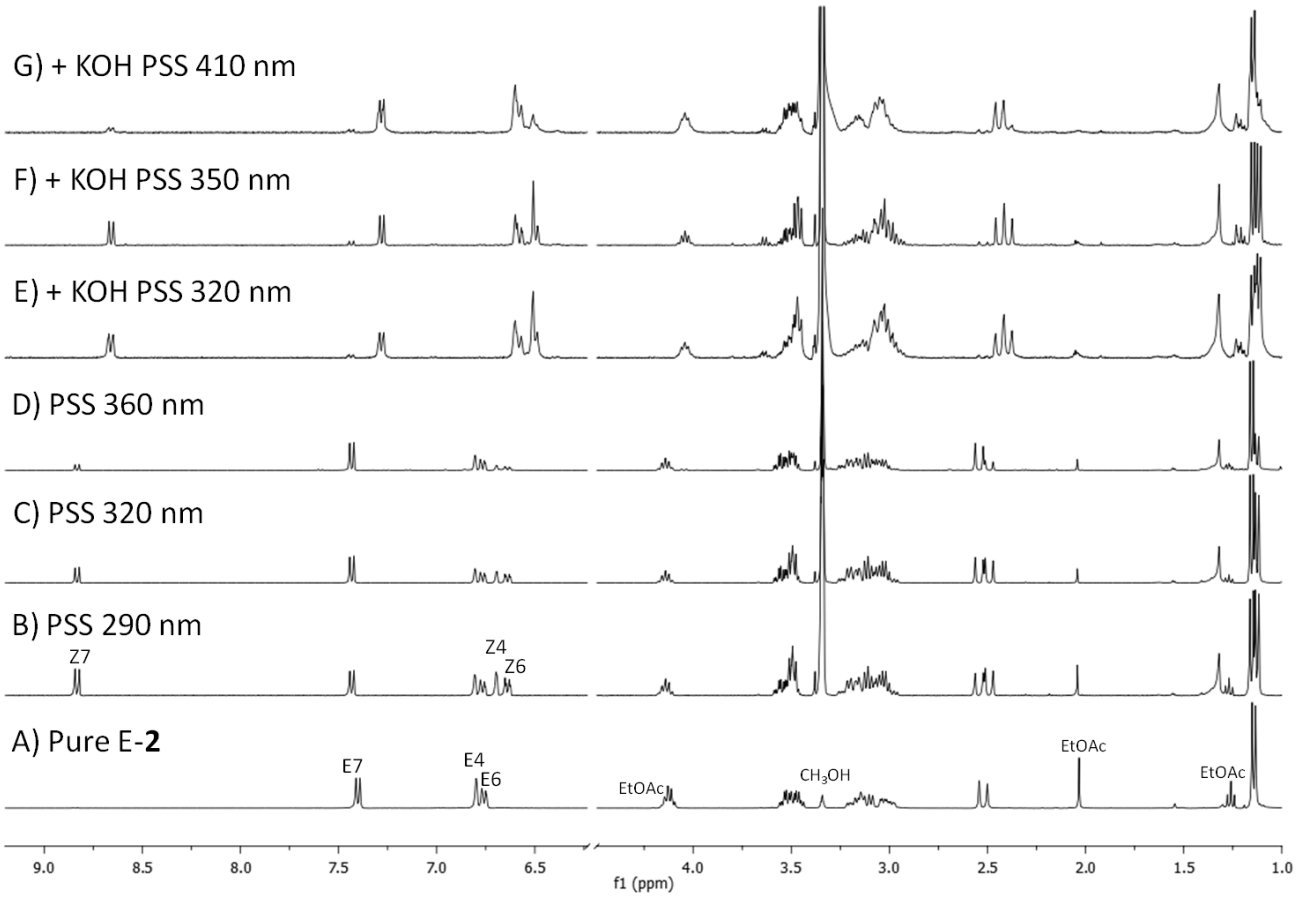

Figure SI-6. Comparison of ^{1}H NMR spectra of compound **2** as pure E isomer in methanol-d_4 and the photostationary states obtained by irradiation of the same solution at 290, 320, and 360 nm in neutral form and at 320, 350 an 410 nm in anionic form (generated by addition of an excess KOH).

References

(1) Schapiro, I.; Gueye, M.; Paolino, M.; Fusi, S.; Marchand, G.; Haacke, S.; Martin, M. E.; Huntress, M.; Vysotskiy, V. P.; Veryazov, V.; et al. Synthesis, Spectroscopy and QM/MM Simulations of a Biomimetic Ultrafast Light-Driven Molecular Motor. *Photochem. Photobiol. Sci.* **2019**, *18* (9), 2259–2269. https://doi.org/10.1039/C9PP00223E.

(2) Sheldrick, G. M. A Short History of SHELX. *Acta Crystallogr. Sect. A* **2008**, *64* (1), 112–122. https://doi.org/10.1107/S0108767307043930.

(3) Sheldrick, G. M. Crystal Structure Refinement with SHELXL. *Acta Crystallogr. Sect. C Struct. Chem.* **2015**, *71* (Md), 3–8. https://doi.org/10.1107/S2053229614024218.

(4) Rossi Paccani, R.; Donati, D.; Fusi, S.; Latterini, L.; Farina, G.; Zanirato, V.; Olivucci, M. Toward a Stable α-Cycloalkyl Amino Acid with a Photoswitchable Cationic Side Chain. *J. Org. Chem.* **2012**, *77* (4), 1738–1748. https://doi.org/10.1021/jo2022263.

(5) Sampedro, D.; Migani, A.; Pepi, A.; Busi, E.; Basosi, R.; Latterini, L.; Elisei, F.; Fusi, S.; Ponticelli, F.; Zanirato, V.; et al. Design and Photochemical Characterization of a Biomimetic Light-Driven Z/E Switcher. *J. Am. Chem. Soc.* **2004**, *126* (30), 9349–9359. https://doi.org/10.1021/ja038859e.

(6) Pronk, S.; Páll, S.; Schulz, R.; Larsson, P.; Bjelkmar, P.; Apostolov, R.; Shirts, M. R.; Smith, J. C.; Kasson, P. M.; van der Spoel, D.; et al. GROMACS 4.5: A High-Throughput and Highly Parallel Open Source Molecular Simulation Toolkit. *Bioinformatics* **2013**, *29* (7), 845–854. https://doi.org/10.1093/bioinformatics/btt055.

(7) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *J. Am. Chem. Soc.* **1996**, *118* (45), 11225–11236. https://doi.org/10.1021/ja9621760.

(8) Aquilante, F.; Autschbach, J.; Carlson, R. K.; Chibotaru, L. F.; Delcey, M. G.; De Vico, L.; Fdez. Galván, I.; Ferré, N.; Frutos, L. M.; Gagliardi, L.; et al. Molcas 8: New Capabilities for Multiconfigurational Quantum Chemical Calculations across the Periodic Table. *J. Comput. Chem.* **2016**, *37* (5), 506–541. https://doi.org/10.1002/jcc.24221.

(9) Ponder, J. W.; Richards, F. M. An Efficient Newton-like Method for Molecular Mechanics Energy Minimization of Large Molecules. *J. Comput. Chem.* **1987**, *8* (7), 1016–1024. https://doi.org/10.1002/jcc.540080710.

(10) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; et al. Gaussian 09. Gaussian Development Version Revision H.38: Wallingford CT 2009.