

Benzylidene-Oxazolones as Molecular Photoswitches

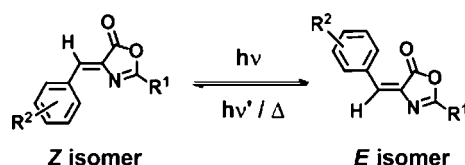
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ABSTRACT



The synthesis and photochemical study of a family of molecular switches inspired by the green fluorescent protein (GFP) chromophore is presented. These compounds can be easily synthesized, and their photophysical properties may be tuned. Due to their efficient photoisomerization and high stability, these compounds can be switched on/off by using light and heat or light with different wavelengths.

Different stimuli have been used recently to induce a mechanical movement at the molecular level.¹ In particular, the use of light has been extensively used to provide a rapid and noninvasive way to control movement.^{2,3} Different types of systems capable of *E/Z* photoisomerization have been already used as molecular switches in order to control diverse properties.^{4–6} Among the several photochemical switches developed, systems based on azobenzene have been extensively used in biological applications⁷ and photomodification of polymers⁸ and to induce photomechanical movements in liquid crystals,⁹ just to mention some examples. Azobenzene derivatives feature very interesting properties such as strong light absorption, efficient photoisomerization, and a great change in the distance between the extremes of the molecule after isomerization.

Moreover, a vast diversity of azobenzene derivatives are synthetically accessible and a detailed knowledge of the relation between substitution and properties is available.

Chiral diarylidene, which provide very different structures and properties, have been also extensively studied.^{10,11} In these compounds, a single isomerizable bond allows for a preferential direction of isomerization. Thus, these compounds can act as molecular rotors or motors. In spite of these appealing properties as effective switches, azobenzene derivatives or chiral diarylidene are just examples of a whole family of compounds capable of performing controlled *E/Z* isomerization. Consequently, the preparation of switches that differ from these structures in properties such as size, shape change, wavelength of absorption, polarity, and isomerization mechanism represent an attractive research target. This could, in turn, expand the applicability of the switch concept to different and increasingly complex molecular environments.

To help in the development of new prototypes, inspiration from Nature can provide useful insights. For instance, the retinal protonated Schiff base (PSB) chromophore of rhodopsins¹² constitutes an example of a very efficient *E/Z* switch shaped by biological evolution. In fact, several families of Rh-based photoswitches have already been

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synthesized and their properties as photoswitches have been reported.^{13–19} These systems have been also used to photocontrol peptide conformations.^{20–22}

Another example of biomolecules that undergo photo-induced *Z/E* isomerizations is the chromophore of the green fluorescent protein (GFP), from *Aequorea victoria* jellyfish. GFPs are intrinsically fluorescent molecules whose optical properties are determined by a photoexcitable green-light emitter chromophore.²³ However, several studies have revealed that the GFP chromophore also undergoes nonradiative processes when irradiated with light, such as a *Z/E* photoisomerization, which contributes to reducing the luminescence quantum yield.^{24–27} Also, different modifications of the imidazoline GFP chromophore have been also reported to show photophysical and photochemical relevant processes.^{25,28,29} Interestingly, well-known intermediates in the synthesis of GFP derivatives such as the oxazolone analogs have been only slightly explored in terms of their photoswitching ability. Although the photoisomerization of benzylidene lactones has been known for some time,^{30,31} only recently have some reports on the photophysical properties of these compounds appeared, with a focus on the fluorescence and its applications.^{32–34} Thus, we report herein the synthesis

and photochemical study of oxazolone analogs of the GFP chromophore to test their ability to act as efficient molecular photoswitches.

The general structure of synthesized compounds is shown in Figure 1, together with the GFP chromophore. The main difference between them is that the GFP chromophore and its derivatives present an amide function in the five-membered ring (dihydroimidazolone), while the compounds that we have studied have an ester function instead (5(4*H*)-oxazolones). The structure of the GFP chromophore can be easily achieved from the synthesized structures through a single reaction step.²⁵ The synthesis of the new photoswitches with the structure based on the GFP chromophore takes place under the classical conditions for the formation of azalactones,³⁵ which is represented in Scheme 1. Using this methodology a number of photoswitches can be easily synthesized (Table 1). In all cases, the *Z* isomer was the only product detected in the reaction crude. The configuration assignment was made on the basis of X-ray diffraction data for **2d** (see Supporting Information (SI) for details).

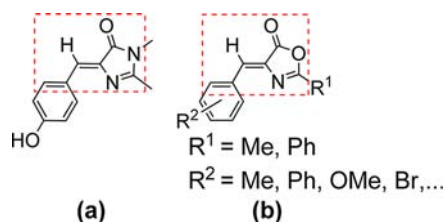


Figure 1. GFP chromophore (a) and oxazolone derivatives (b).

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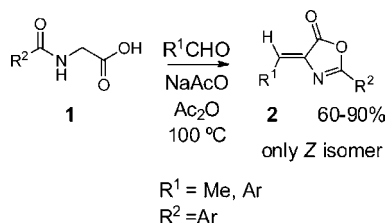
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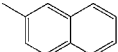
Scheme 1. Synthesis for GFP-Based Photoswitches



Although this methodology can be used to prepare a wide range of products, the use of certain benzaldehyde derivatives, such as 2-carboxy- or 2-cyanobenzaldehyde, and ketones is prevented. Next, the UV–vis spectra for all the synthesized compounds were measured. The values for the maximum wavelengths and the extinction coefficients of the bands found for each compound in acetonitrile are displayed in Table S1. Interestingly, these compounds

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Table 1. Synthesized Molecular Switches

entry	R ₁	R ₂	switch	yield
1	Ph	Ph	2a	85%
2	Ph	<i>p</i> -MeOPh	2b	60%
3	Ph	<i>p</i> -BrPh	2c	72%
4	Me	<i>o</i> -MeOPh	2d	82%
5	Me	Ph	2e	80%
6	Me	<i>p</i> -NO ₂ Ph	2f	90%
7	Me		2g	61%

show a maximum of absorption around 350 nm, though the presence of electron-donating groups provokes a red shift of the band. Thus, **2b** absorbs in the visible. This is indeed a very useful feature for a photoswitch, as low-energy light compatible with complex environments such biological media could be used. Besides, the UV–vis spectra of the compounds were measured in diverse solvents with varying polarity. Acetonitrile and chloroform were used to test if there was any solvent effect. The spectrum for **2e** is shown in Figure S1. It should be noted that the maximum absorption wavelength of the band only suffers a slight (5 nm) red shift when changing from a polar (acetonitrile) to a nonpolar (chloroform) solvent. However, in general terms, we can consider that the UV spectrum of these compounds remains almost the same, so they can be used in different environments without large modifications in the photophysical properties.

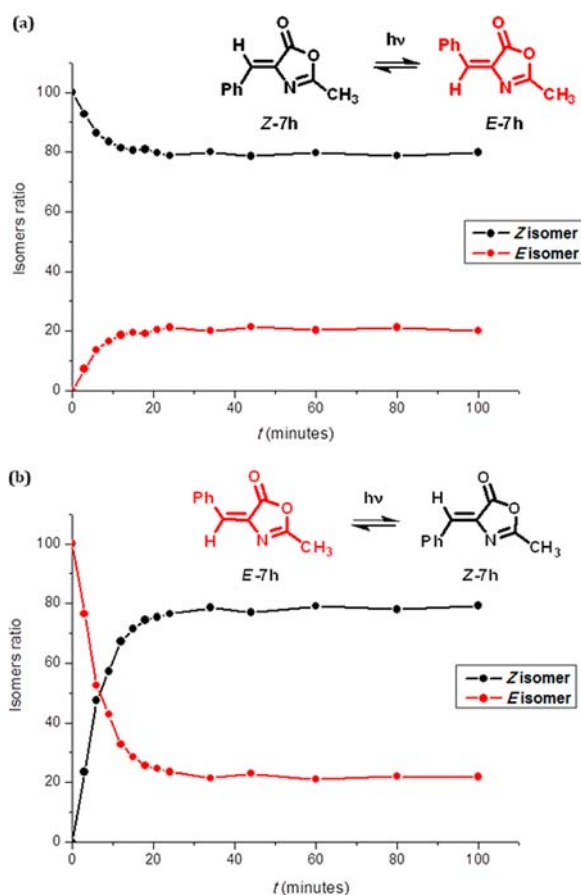
Analysis of the UV–vis spectra of the photoswitches showed that, for irradiating the different compounds, a Pyrex filter can be used to avoid radiation below 290 nm. Thus, as the light source, a 125-W medium-pressure Hg lamp was used. The results for the irradiation in an immersion well reactor of 0.01 M solutions of a selection of photoswitches in acetonitrile are shown in Table 2. The irradiation process of each compound was followed by ¹H NMR, at different time intervals, until the photostationary state (PSS) was reached, since *Z* and *E* isomers have distinctive ¹H NMR signals (see SI). Integration of the ¹H NMR signals corresponding to each isomer allowed us to know the isomer ratio at a given irradiation time. Depending on the absorption bands, the irradiated mixture took from 1 to 3 h to reach the PSS. The resulting mixture of isomers could be separated by flash chromatography on silica gel, using hexane/ethyl acetate (10:1) as eluent.

Then, we studied the kinetics of the isomerization reaction. For this purpose, we prepared in two distinctive Pyrex NMR tubes two 0.07 M solutions in CDCl₃, one for each isomer of **2e**. Afterward, both NMR tubes were irradiated in a 125-W medium-pressure Hg lamp and a Pyrex filter, until both solutions reached the PSS. As the samples were directly dissolved in CDCl₃, it was easy to follow the photoisomerization reaction by ¹H NMR at short irradiation intervals. Therefore, ¹H NMR spectra of the two

Table 2. Photostationary State

entry	R ₁	R ₂	switch	ratio <i>Z/E</i>
1	Ph	Ph	2a	75/25
2	Ph	<i>p</i> -MeOPh	2b	83/17
3	Ph	<i>p</i> -BrPh	2c	75/25
4	Me	<i>o</i> -MeOPh	2d	64/36
5	Me	Ph	2e	80/20
6	Me	<i>p</i> -NO ₂ Ph	2f	83/17
7	Me		2g	83/17

samples were performed every 3 min with the aim of tracking the isomerization process of the compounds **Z-2e** and **E-2e**. Representing the isomer ratio vs the irradiation time, we obtained the two graphs that are shown in Figure 2, (a) corresponding to the sample with 100% of **Z-2e** isomer at the beginning of the reaction, and (b) corresponding to the sample with 100% of **E-2e** isomer at *t*_{irrad} = 0 min.

**Figure 2.** Isomers ratio vs the irradiation time starting from the (a) *Z* isomer and (b) *E* isomer of compound **2e** after irradiation with a 125-W medium-pressure Hg lamp and a Pyrex filter.

As can be seen, the PSS is rapidly reached (*ca.* 30 min) and the isomer ratio is independent from the starting isomer (*Z* or *E*). On the other hand, from the linear

relationship between the first points of each graph of Figure 2 (representing $\ln [A_0]/[A]$ vs irradiation time with a linear fit), we can calculate the values for the kinetic constants of each process and, therefore, the value of the relative kinetic constant that relates the speed of both processes. In this particular case, if $k_{Z \rightarrow E} = 1$, then $k_{E \rightarrow Z} \approx 4.5$, which means that the $E \rightarrow Z$ isomerization process is 4.5 times faster than the $Z \rightarrow E$ reaction. Besides, when both samples reached the PSS, we proceeded to heat them at 50 °C in the dark for several hours, to see if the isomer ratio was altered. By studying the $^1\text{H NMR}$ spectra of the heated samples, we deduced that at that temperature the isomer ratio remained unchanged. This implies that these photo-switches are thermally stable at 50 °C and can be operated by light without the interference of thermal processes. Using deuterated toluene, we warmed up the samples at 100 °C. This way, we observed that the Z isomer remained unchanged when heated for long periods of time. However, the E isomer slowly reverted to the Z isomer, and after heating for 776 h, this reversion was fully achieved. Consequently, we can use this property to tune the switch behavior, obtaining mixtures of isomers rich in one isomer or another depending on whether we use light or heat.

We then explored the effect of the wavelength of irradiation in the switching process. Figure 3 shows the UV spectra for both isomers of **2e**.

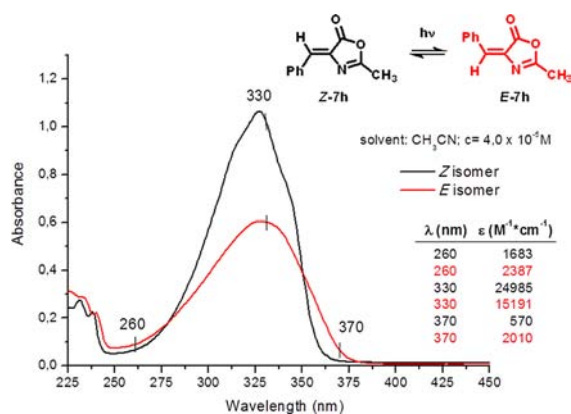


Figure 3. UV–vis spectra for the Z and E isomers of compound **2e**.

It can be seen that there are slight differences in the shape of their absorption bands. Thanks to this effect, we could selectively irradiate in different regions of the absorption spectrum of each isomer with monochromatic light to obtain different isomer ratios at the PSS depending on the irradiation wavelength (SI). We first irradiated a solution of each isomer of **2e** at 330 nm until the PSS

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was reached. As shown in Figure 3, at 330 nm the Z isomer has a stronger absorption. Consequently, a PSS richer in the E isomer was expected. The progress of the reaction was followed by $^1\text{H NMR}$, and the isomer ratio at the PSS was found to be 43% (Z)/57% (E) independently of the starting isomer. Then we selected an irradiation wavelength from the end of the band (370 nm). At this wavelength, the E isomer presented stronger absorption than the Z isomer. The equilibrium was displaced toward the formation of Z , being the main isomer at the PSS, with a value of 83% (Z)/17% (E). Finally, we selected a wavelength from the beginning of the band (260 nm). At this wavelength, the E isomer absorbed more than the Z , but the absorption for both isomers was really low. This means that the $E \rightarrow Z$ isomerization was favored over the $Z \rightarrow E$ reaction. Consequently we found a mixture of isomers rich in Z , although we were not able to reach the PSS due to low reaction rates. Therefore, we can obtain mixtures of isomers rich in Z at the beginning and end of the absorption band (260 and 370 nm), while mixtures of isomers rich in E are afforded when irradiating at the maximum of the band (330 nm).

To check the photoisomerization efficiency we measured the quantum yield of the reaction using the two isomers of **2e** to determine the photoisomerization quantum yield for both the $Z \rightarrow E$ reaction and the $E \rightarrow Z$ process. Using *trans*-azobenzene³⁶ as the actinometer (SI), we obtained 0.25 ± 0.01 for the $Z \rightarrow E$ process and 0.11 ± 0.02 for the $E \rightarrow Z$ isomerization. Thus, these prototypes of molecular switches can be considered quite efficient, as the measured Φ values for the two isomers of this switch are similar to the values obtained for the photoisomerization of analogous compounds based on the GFP chromophore.³⁷

We have shown that an efficient photoswitching process can be achieved by using oxazolone analogs of the GFP chromophore. The easy and versatile synthesis allows for the preparation of derivatives with a different substitution pattern that can be used to tune the photophysical properties. The main isomer can be controlled not only by using light or heat as the stimulus but also through careful selection of the wavelength of irradiation. Further efforts to fully understand the photophysics of the process and to use these switches in a complex system are currently underway.

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Supporting Information Available. Synthetic procedures, characterization data and CIF file for **2d**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.