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When light and acid play *tic-tac-toe* with a nine-state molecular switch.

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ABSTRACT: Combining different molecular switching functions in a single molecule is a simple strategy to develop commutable molecules featuring more than two commutation states. The present study reports on two molecular systems consisting of two indolino-oxazolidine (Box) moieties connected to an aromatic bridge (phenyl or bithiophene) by ethylenic junctions. Such systems, referenced as BiBox, are expected to show up multiaddressable and multiresponsive behaviors. On one hand, the oxazolidine ring opening/closure of Box moieties can be addressed by chemical stimuli, and on the other hand, the trans-to-cis isomerization of the ethylenic junctions is induced by visible light irradiation (with a thermal back conversion). NMR and UV-visible spectroscopies allowed to characterize up to nine out of the ten theoretically expected commutation states as well as to measure the kinetics of the interconversions. The open/closed states of the Box moieties also affected the emission properties which were determined by steady state fluorescence spectroscopy.

INTRODUCTION

Molecular switching defines the commutation behavior of a single molecule between two states upon a specific stimulus which can be chemical, photochemical or electrochemical. Among those different possibilities, light driven switching molecules are of great interest in a wide range of fields due to localized and usually fast commutation processes. The major interest resides in the difference between the molecular structure of the switching states, and so chemical or physical properties like absorption, fluorescence, or nonlinear optic (NLO) activity.¹ This concept is the basis of many active research fields such as light modulated materials², molecular machines³ or optogenetics⁴ based on azobenzene, spiropyran or diarylethene switching properties, among the most studied families.

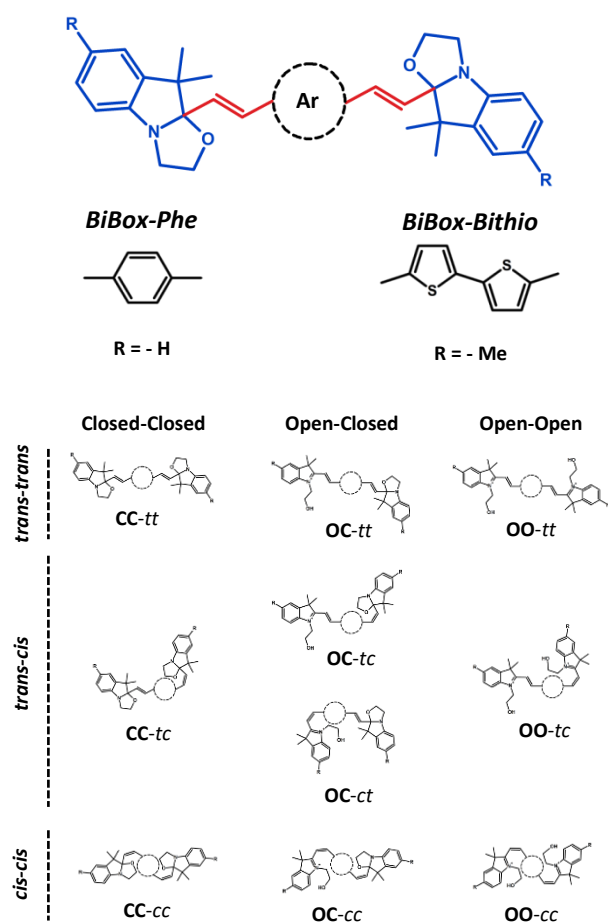
Recently, the indolino[2,1-b]oxazolidine (Box) family has triggered intensive research interest due to its multi-addressable nature⁵. Its commutation behavior consists in the opening of the oxazolidine ring by using indifferently acid addition⁶, UV light irradiation⁷ or electrochemical

stimulation.^{8,9} The Box closing can then be induced by base treatment or electrochemically, leading to a fully reversible two-state molecular switch. If the Box moiety is linked to an aromatic chromophore by an ethylenic junction, both functions become conjugated when the Box is in its open state. As the conjugated system is larger, the energy of the main transition is lower and characterized by an intense absorption band in the visible range.⁵ This important variation of physico chemical properties between the commutation states have recently conducted to clever and unusual applications as water-jet rewritable paper development based on the Box solvatochromism properties.^{10–13} Also, the indoleninium moiety obtained after the Box opening is an electron withdrawing group bringing a higher NLO response which has been extensively described experimentally and theoretically in the literature.^{14–17} At the opposite, examples of fluorescence modulation of organic fluorophores by closing or opening the Box moiety appear somehow limited. To the best of our knowledge, only their use as chemical probes for the detection of galactosidase¹⁸ or thiophenols¹⁹ in solution

was reported, as well as the modulation of fluorescence of a tetraphenylethene covalently linked to a Box moiety.²⁰

In addition to the opening/closing reactions, the trans-cis isomerization of the ethylenic junction allowed to extend to four the number of commutation states.^{21,22} Indeed, the trans isomer is thermodynamically favored and visible light irradiation induces its isomerization towards the cis conformer, whatever the closed or open state of the Box moiety. Then, the association of Box and ethylenic junction, their switching properties and their potential fluorescence modulation let envisage building multiaddressable compounds with multiresponsive behavior upon specific stimulus.

We then report here on advanced molecular switches consisting of two Box moieties linked by an aromatic bridge referred here as BiBox. Easily accessible due to their simple preparation^{23,24}, two aromatic bridges were selected: 1,4-phenylene (BiBox-Phe) and 2,2'-bithiophene (BiBox-Bithio) differing by size and aromaticity (Scheme 1).



Scheme 1. Structure of the studied BiBox compounds, and the ten theoretically expected isomers.

Taking into account the two Box moieties and the two ethylenic junctions, up to ten commutation states are theoretically expected. Here, the Box state is referred with

C (Closed) or O (Open) and the configuration of the ethylenic junction is denoted as t and c for trans and cis, respectively (Scheme 1). One can first consider the two Box moieties in the same closed (CC) or open (OO) state. Three different isomers are possible for these two states, depending on the ethylenic junction trans-cis configuration which can be all-trans (-tt), trans-cis (-tc) or all-cis (-cc). When only one Box is open, four isomers can be drawn due to the molecule asymmetry. The characterization of the different commutation states as well as their switching behavior upon light irradiation or chemical stimuli have been studied by ¹H NMR spectroscopy and UV-Visible spectrophotometry. Fluorescence properties have also been investigated and discussed based on related Box compounds.

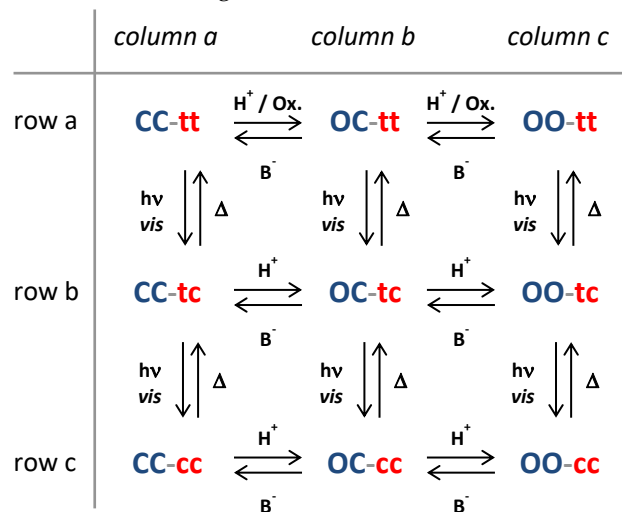
RESULTS AND DISCUSSION

Effect of acid and oxidant on the Box opening in the CC-tt state. (Scheme 2, row a)

UV light irradiation was reported as being neither an efficient nor a selective stimulus to induce the Box moiety opening in such system.²⁴ Then, chemical stimuli were considered as a stepwise way to obtain OC-tt and OO-tt species from the most thermally stable CC-tt in solution. The hydrochloric acid (DCl + D₂O) and oxidant (NOSbF₆) titrations of BiBox-Phe solutions in acetonitrile-d₃ were followed by ¹H NMR spectroscopy. The spectrum of the initial CC-tt state is characterized by multiplets between 3.3 and 3.9 ppm (Figure S1) arising from the methylenic protons of the constrained oxazolidine moiety. Up to 1.0 eq. of acid or oxidant, the coloration of the solution associated with the appearance of the Open-Closed (OC-tt) signals was observed concomitantly with the decrease of the intensity of the CC-tt signals. Due to the asymmetric structure of OC-tt, its NMR spectrum is complex and consists of a combination of a set of resonances characteristic of the closed Box, very similar to the CC-tt spectrum and new signals generated by the formation of the indoleninium moiety. This latter is well-characterized by two triplets at 4.0 and 4.7 ppm assigned to the methylenic protons of the open oxazolidine ring. Pushing further the stimulation by acid or oxidant allowed the progressive conversion of OC-tt to the symmetrical OO-tt state featuring two NMR triplets (I = 2 x 2H) at 4.0 and 4.8 ppm characteristic of the pendant alkyl chain protons of the open oxazolidine rings (Figure S1).

Concentration profiles were derived from ¹H NMR spectra by integrating characteristic signals for the three states (Figure 1 a and b). The full conversion of CC-tt to OO-tt was strongly dependent of the nature of the stimulation. Indeed, 2.0 eq. of oxidant were required while 3.0 eq. of acid had to be added in order to reach 100% of OO-tt. Such difference may be explained by a higher equilibrium constant for the oxidation process than for the protonation. In this context, it was not surprising to detect a persistency of CC-tt in the solution after the addition of 2.0 equivalents of acid whereas it was almost consumed after 1.0 equivalent of oxidant. The quantification of each state

upon the addition of acid or oxidant highlights the preferential opening of one Box then the opening of the second one. However, the process is not perfectly selective, as it was not possible to generate 100% pure OC-tt. This latter was always found in mixture with CC-tt and OO-tt with a ratio reaching 80%.



Scheme 2. Schematic representation of interconversion processes.

The titration with acid of BiBox-Phe was also followed by UV-visible spectrophotometry (Figure 1c). The CC-tt state absorbed only in the UV range and its absorption profile featured a pronounced vibronic structure ($\lambda_{\text{max}} = 290 \text{ nm}$). Upon acid addition, the solution turned yellow, associated with the formation of OC-tt absorbing in the visible range with a Gaussian shaped absorption band centered at 425 nm. The opening of the second Box moiety implied a bathochromic shift (34 nm) and the absorption profile of the corresponding OO-tt form was dominated by an intense absorption band with a discrete vibronic structure. Compared to the BiBox-Bithio compounds (bathochromic shift = 17 nm),²⁴ the absorption spectra of BiBox-Phe in OC-tt and OO-tt states are more distinguishable due to the largest shift of their absorbance maxima.

Finally, the initial CC-tt state was restored by treatment of OO-tt with vapor of base (triethylamine). Then, the fully reversible switching of the system BiBox-Phe (CC-tt \rightarrow OC-tt \rightarrow OO-tt) induced by chemicals and its bimodal commutation ability were here demonstrated and showed a similar addressable selectivity between both Box units when compared with the BiBox-Bithio.²⁴

Emission properties of CC-, OC- and OO-tt

Even though the absorption spectra of BiBox-Phe in OC-tt and OO-tt states differ more ($\Delta\lambda = 34 \text{ nm}$) than for the two corresponding states of BiBox-Bithio ($\Delta\lambda = 17 \text{ nm}$), they remain close. Thus, fluorescence was addressed as a new way to decipher the open/closed states with a better sensitivity.

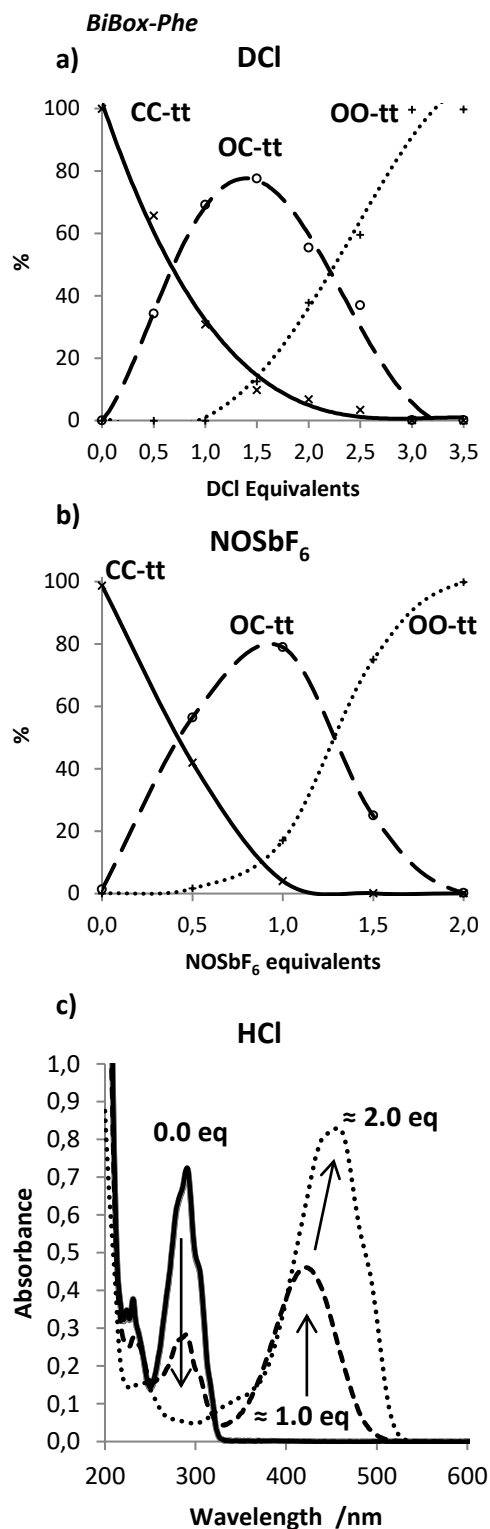


Figure 1. Titration of BiBox-Phe in acetonitrile-d₃ with a) acid, b) oxidant followed by NMR spectroscopy, and c) acid titration in acetonitrile followed by UV-Visible spectroscopy.

As only few examples of such Box properties have been reported,^{15,18-20} the steady-state emission of the BiBox systems in acetonitrile solutions was investigated as a function of the open or closed state of the Box moieties. For a complete overview and as a purpose of comparison,

two simple Box molecules (compounds 1 and 2, Figure 2) were also studied. The closed state of 1 in acetonitrile presented two major gaussian shaped absorption bands in the UV range with maxima located at 237 and 285 nm, the one at higher energy being the most intense (Figure 2a). Upon an excess of hydrochloric acid ($\text{HCl} + \text{H}_2\text{O}$), the full conversion to the corresponding open form was reached featuring a similar absorption spectrum with broader absorption bands at slightly higher energy (232 and 278 nm). Emission properties were hugely driven by the commutation state. Indeed, while an intense fluorescence band was observed at 345 nm for the closed state of 1, the emission of the open form was very low. The quantum yields of fluorescence with 290 nm excitation wavelength were 22.5% and < 0.1% for closed and open states respectively, hence highlighting an on-off fluorescent molecular switch commutable by acid stimulus. This difference in quantum yield may be related to the very constrained closed form structure, preventing from non-radiative desexcitation. The compound 2 in its closed state only absorbed in the UV range with a maximum at 283 nm (Figure 2b). Previous studies showed that a $\pi \rightarrow \pi^*$ electronic transition of the vinyl-thiophene system was responsible of this absorption band.²¹ The ring-opening with acid allowed for electronic conjugation across the whole flat structure leading to a main transition at lower energy, typically in the visible range here located at 422 nm. Only low intensity emission was detected for the two states with 0.1 % quantum yield of fluorescence for the closed state, while the open one was largely lower. Such a difference in quantum yields between compounds 1 and 2 in the closed state is explained by more degrees of freedom brought by the vinyl-thiophene moiety allowing non radiative de-excitation especially by rotation around the ethylenic junction and possibly trans/cis isomerization. For compound 2, very similar Stokes shifts were found for closed and open states (between 6000 and 6700 cm^{-1}) and emission bands were centered at 350 and 564 nm respectively.

The emission spectrum of the CC-tt state of BiBox-Bithio featured an intense band in the blue region, mirrored to the corresponding absorption band, thus displaying a net vibronic structure (Figure 3a). The quantum yield of fluorescence was 20.7% ($\lambda_{\text{exc}} = 370\text{nm}$) and the maximal emission band was at 450 nm. The ring-opening of one or two Box moieties induced a shift of the emission band towards the red region with a maximum at 662 nm for both OC-tt and OO-tt forms. However, the quantum yields of fluorescence fell to 1.1% and 3.6% respectively ($\lambda_{\text{exc}} = 530\text{ nm}$). This switching of the emission properties over consecutive opening of the two Box moieties for BiBox-Bithio in solution can be appreciated by naked eyes as shown on Figure 3b. Indeed, the blue fluorescence of CC-tt state was observed under UV irradiation, almost no fluorescence for OC-tt and a bright red emission for OO-tt when these two latter were irradiated in the visible range. The same tendency was observed for BiBox-Phe (Figure 3c) with rather low quantum yields of fluorescence, where CC-tt was the most emissive state, OC-tt the lowest and OO-tt

intermediate with 1.1%, <0.1% and 0.4% respectively. Nevertheless, this system features an interesting emission property as for each commutation state the fluorescence band fell in the 530 – 560 nm region. This characteristic is due to a large Stokes shift for CC-tt form, ca. 16 000 cm^{-1} versus roughly 5 000 cm^{-1} for OC-tt and OO-tt. This huge shift and the gaussian shaped emission band contrary to the structured absorption band suggest an important molecular reorganization at the excited state. Regarding the position and the shape of the emission band, a distortion around the divinylbenzene aromatic bridge is likely to explain this phenomenon. This is illustrated by the very different emission behaviors of BiBox-Phe and -Bithio in CC-tt state where ground and excited state structures of the latter should be similar. These observations are in agreement with the emission behavior of related compounds 1 and 2, having only one Box moiety.

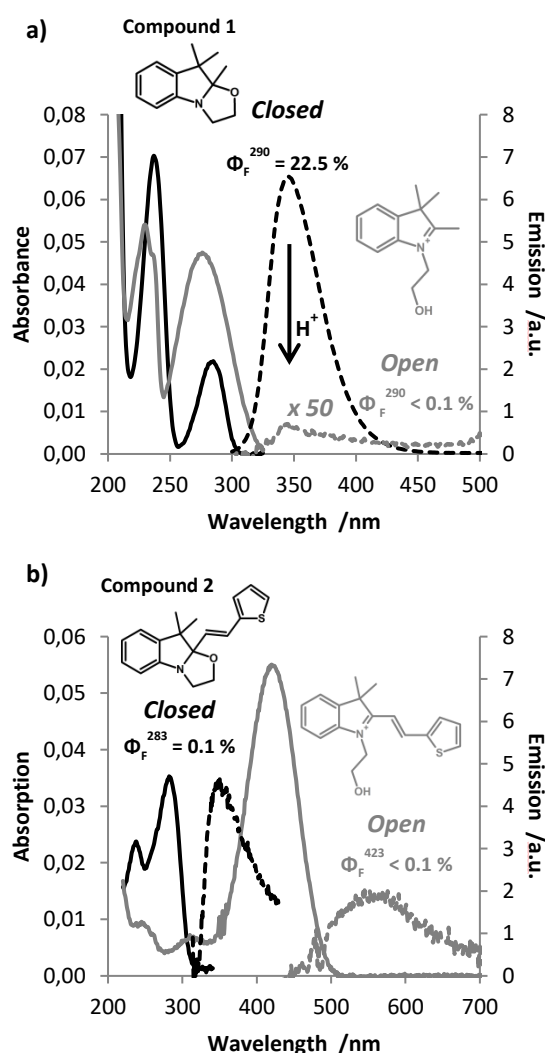


Figure 2. (solid) Absorption and (dotted) emission spectra of compounds (a) 1 and (b) 2 in closed and open states. Excitation wavelengths : 290 nm (compound 1) : 283 and 423 nm (closed and open compound 2 respectively).

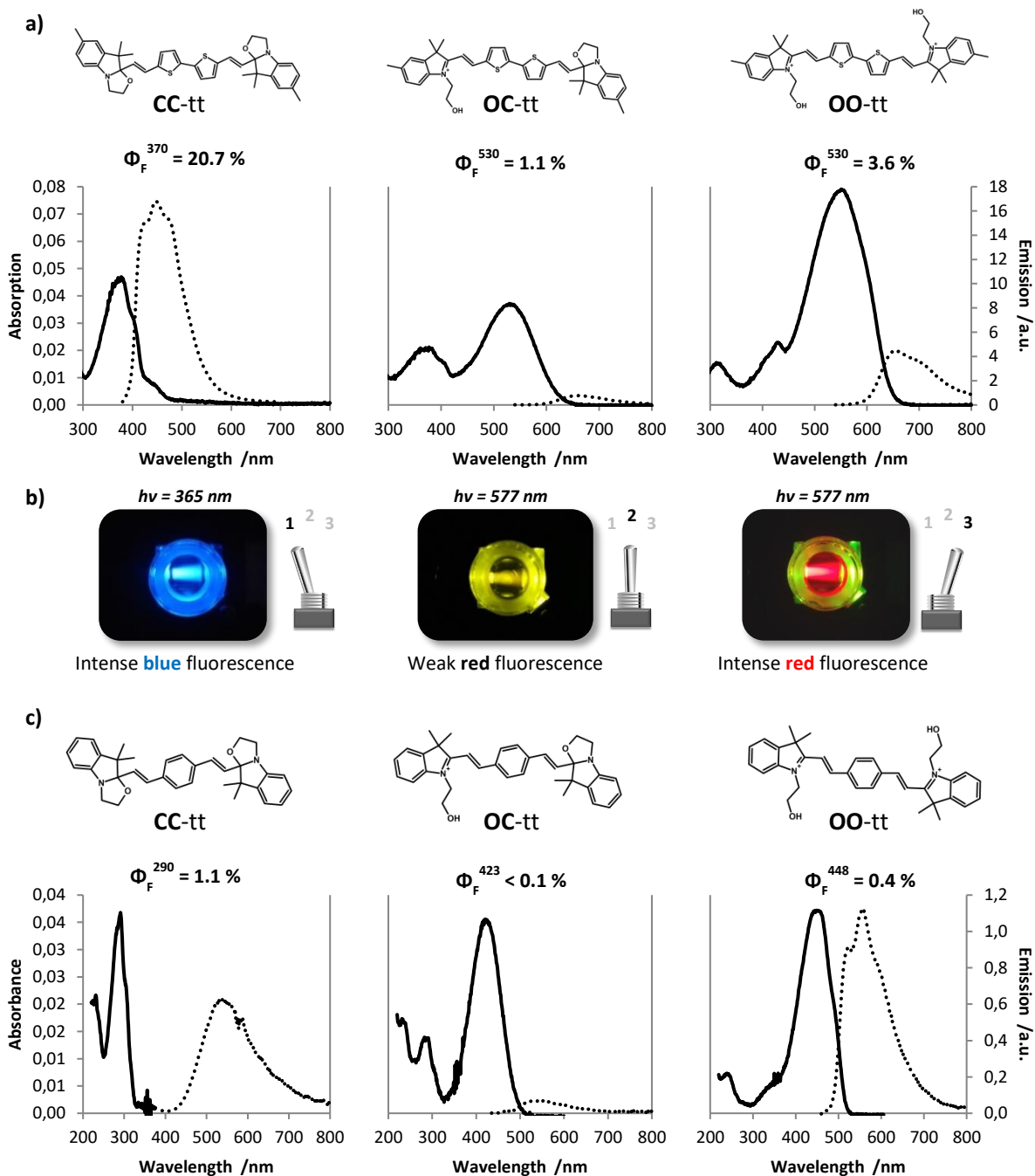


Figure 3. (solid) Absorption and (dotted) emission spectra at $\lambda_{\text{exc}} = 370$ nm (CC-tt) and 530 nm (OC-tt and OO-tt) for (a) BiBox-Bithio and $\lambda_{\text{exc}} = 290$ nm (CC-tt), 423 nm (OC-tt) and 448 nm (OO-tt) for (c) BiBox-Phe. (b) Raw pictures of the corresponding BiBox-Bithio solutions under irradiation at 365 nm (CC-tt) and 577 nm (OC- and OO-tt).

Effect of visible light irradiation on the OO-tt state. (Scheme 2, column c)

An excess of acid (TFA vapor) was added to fresh solutions of CC-tt to produce 100% of OO-tt in chloroform. Then, visible light irradiation was applied to induce the photoisomerization of the ethylenic junctions of the BiBox systems. Irradiation of the OO-tt state of BiBox-Phe (BiBox-Bithio) with 436 nm (546 nm) light induced a rapid decrease of the absorbance of the main band from

0.80 to 0.47 (0.81 to 0.17) and a hypsochromic shift of 35 nm (22 nm) was observed (Figure 4).

The structural changes induced by light irradiation were also followed by NMR spectroscopy, to characterize the structures of the photogenerated states and the kinetics of their formation. Between each successive periods of irradiation, ^1H NMR spectra were recorded underlining the decrease of OO-tt signals and the appearance of a first set of resonances (OO-tc), then a second one (OO-cc).

The cis configuration of the ethylenic junctions was nicely evidenced by the vicinal coupling constant of ~ 13 Hz for the doublets of the ethylenic protons, a smaller value than that measured for the trans isomers (16-17 Hz - Figure S2 to Figure S5). The ^1H NMR spectrum of OO-tc was more complex due to its asymmetric structure, showing four doublet signals for the four ethylenic protons, two in cis configuration and two in trans configuration. In contrast, the ^1H NMR spectrum of the OO-cc state was much simpler due to the recovered molecular symmetry and featured two doublets ($I = 2 \times 2\text{H}$ and $3J \sim 13$ Hz).

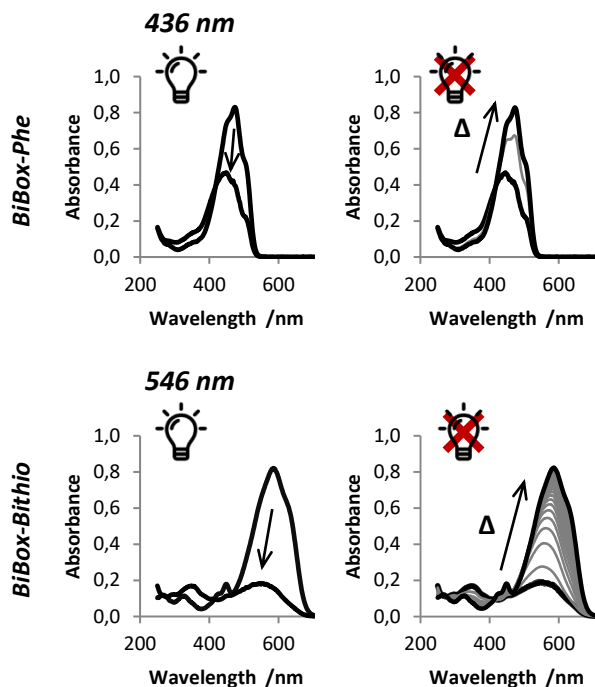


Figure 4. UV-Visible spectra of (top) BiBox-Phe and (bottom) BiBox-Bithio (OO-tt state) in chloroform (left) under visible light irradiation and (right) during thermal relaxation in the dark.

By measuring the peak-intensities of some characteristic signals for the three states, the time-evolution of concentrations was plotted (Figure 5). Upon 436 nm light irradiation, the OO-tt state of BiBox-Phe was isomerized into 50% of OO-tc and 15% of OO-cc at the photostationary state. A higher conversion was not reached as both photoproducts also absorbed at 436 nm. The same trends were observed with BiBox-Bithio. Irradiation with 546 nm light produced only 25 % of OO-tc and 5% of OO-cc at PSS.

When the photostationary state (PSS) was attained, irradiation was cut-off and the thermal evolution was recorded showing a slow reversion. An additional period of irradiation by changing the wavelength of light was then applied to modulate the proportion of each state and the thermal relaxation was again recorded when PSS was reached. For BiBox-Phe submitted to visible light ($\lambda > 495$ nm), the initial OO-tt state was converted into 90% of OO-tc and only 5% of OO-cc was detected. This phenom-

enon can be easily rationalized as only OO-tt absorbed at such long wavelength range, and so the back photoisomerization of OO-tc to OO-tt did not occur. For BiBox-Bithio irradiated with 577 nm light, 40% of OO-tc and 10% of OO-cc states were obtained. It is then noteworthy that a selective and quantitative photo-isomerization can be activated by choosing the adequate excitation wavelength although less efficient with the BiBox-Bithio derivative.

From the NMR spectra acquired during the thermal relaxation, the time-evolution of the concentrations were plotted and fitted with two consecutive processes: $\text{OO-cc} \rightarrow \text{OO-tc} \rightarrow \text{OO-tt}$ characterized by two rate constants (k_1 and k_2 , respectively). To determine if solvent could affect the thermal evolution of the systems, experiments were repeated with acetonitrile solutions (Figures S3 and S5). The set of extracted rate constants of relaxation are gathered in Table 1 a. In all cases, the $\text{cc} \rightarrow \text{tc}$ isomerization was faster than the $\text{tc} \rightarrow \text{tt}$ reaction. This is not surprising as the cis configuration of the double bond led to a more constrained structure, favoring its transformation into the less constrained OO-tc state that relaxed towards OO-tt but from two to three times slower. However, the bithio-phenyl bridge allowed to stabilize greater the cis states as the rate constants were always lower than that measured for the phenyl derivative. Even, a great increase in stability was observed in more polar acetonitrile, while it was exactly the contrary with BiBox-Phe. Add something?

Table 1. Thermal kinetic constants k_1 ($\text{cc} \rightarrow \text{tc}$) and k_2 ($\text{tc} \rightarrow \text{tt}$) of relaxation at 295 K for all-open, all-closed and mixed BiBox states.

a) $\text{OO-cc} \rightarrow \text{OO-tc} \rightarrow \text{OO-tt}$				
BiBox	Solvent	$k_1 / 10^{-4}\text{s}^{-1}$	$k_2 / 10^{-4}\text{s}^{-1}$	k_2/k_1
Phe	ACN	8.45	4.27	0.50
	CDCl_3	6.26	1.98	0.32
Bithio	ACN	0.51	0.16	0.31
	CDCl_3	2.49	1.07	0.44
b) $\text{CC-cc} \rightarrow \text{CC-tc} \rightarrow \text{CC-tt}$				
BiBox	Solvent	$k_1 / 10^{-4}\text{s}^{-1}$	$k_2 / 10^{-4}\text{s}^{-1}$	k_2/k_1
Phe	ACN	0.15	0.06	0.43
	CDCl_3	9.10	2.61	0.29
Bithio	ACN	0.60	0.29	0.48
	CDCl_3	1.18	0.59	0.51
c) $\text{OC-cc} \rightarrow \text{OC-tc} \rightarrow \text{OC-tt}$				
BiBox	Solvent	$k_1 / 10^{-4}\text{s}^{-1}$	$k_2 / 10^{-4}\text{s}^{-1}$	k_2/k_1
Phe	ACN	--	Too fast	/
Bithio	ACN	44.52	0.78	0.02

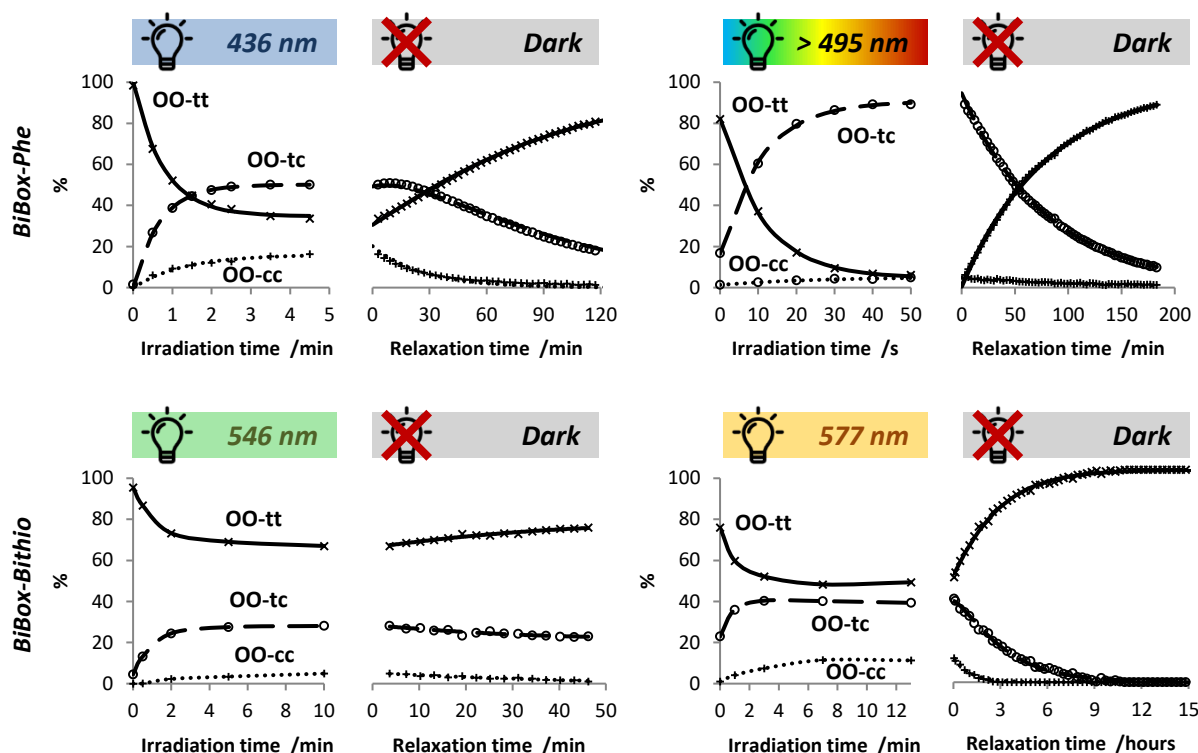


Figure 5. Kinetics of OO-tt photoisomerization towards OO-tc and OO-cc and thermal relaxation for (top) BiBox-Phe and (bottom) BiBox-Bithio in chloroform

Effect of visible light irradiation on the CC-tt state. (Scheme 2, column a)

According to our previous work,²¹ a high trans-to-cis conversion could be obtained when irradiating a simple closed Box derivative with visible light even though it did not absorb in the visible range. Such unusual behavior was explained by the acid/base equilibrium between the closed and open forms in solution which was largely shifted towards the closed species. A quantitative trans→cis photoisomerization was observed following an indirect reaction pathway. Based on these results, the BiBox CC-tt systems were subjected to visible light irradiation (436 nm and 577 nm for BiBox-Phe and -Bithio respectively), to produce the trans-cis and cis-cis isomers (CC-tc and CC-cc).

The reactions were followed by ¹H NMR spectroscopy in acetonitrile and chloroform. In both solvents, the CC-tt signals of BiBox-Phe and BiBox-Bithio slowly decreased upon irradiation while two sets of resonances appeared. Characteristic resonances for CC-tc state were observed i) two doublets for the four benzene protons of the aromatic bridge indicated a non-symmetrical molecule; ii) four doublets (³J = 13.0 Hz and ³J = 16 Hz) proved the cis and the trans ethylenic configurations of the double bonds. The second set of signals was assigned to the CC-cc state with i) unresolved multiplets between 3.1 and 3.6 ppm for the methylene protons; ii) vinylic doublets with ³J = 13.0

Hz ($I = 2 \times 2H$) for the ethylenic protons (Figure S6). As both oxazolidine units were in cis configuration, a symmetrical structure was expected. However, some signals were inequivalent due to specific conformations on each side of the molecule. As previously observed, the cis isomers were not thermally stable leading to the back reaction CC-cc → CC-tc → CC-tt when the light source was turned off. The kinetics of the photochemical and thermal reactions were recorded (Figure 6). As expected, longer irradiation time is necessary to convert CC-tt compared to the OO-tt photoisomerization. Tens of minutes up to several hours allow to reach a PSS when few minutes were enough previously. Also, solvent has a huge influence on the photo conversion and the thermal relaxation. PSS is attained 3 to 5 times faster in chloroform than in acetonitrile for BiBox-Phe and -Bithio respectively. Considering the thermal back reaction, kinetic constants for BiBox-Bithio CC-cc → CC-tc → CC-tt reaction are comparable to what have been found for OO-cc → OO-tc → OO-tt (Table 1 b), where the two successive reactions appear faster in chloroform. However, for BiBox-Phe, cis isomers of the doubly closed isomer are a lot more stable in acetonitrile than in chloroform, as kinetic constants are 40 (k_1) to 60 (k_2) times greater in the latter solvent.

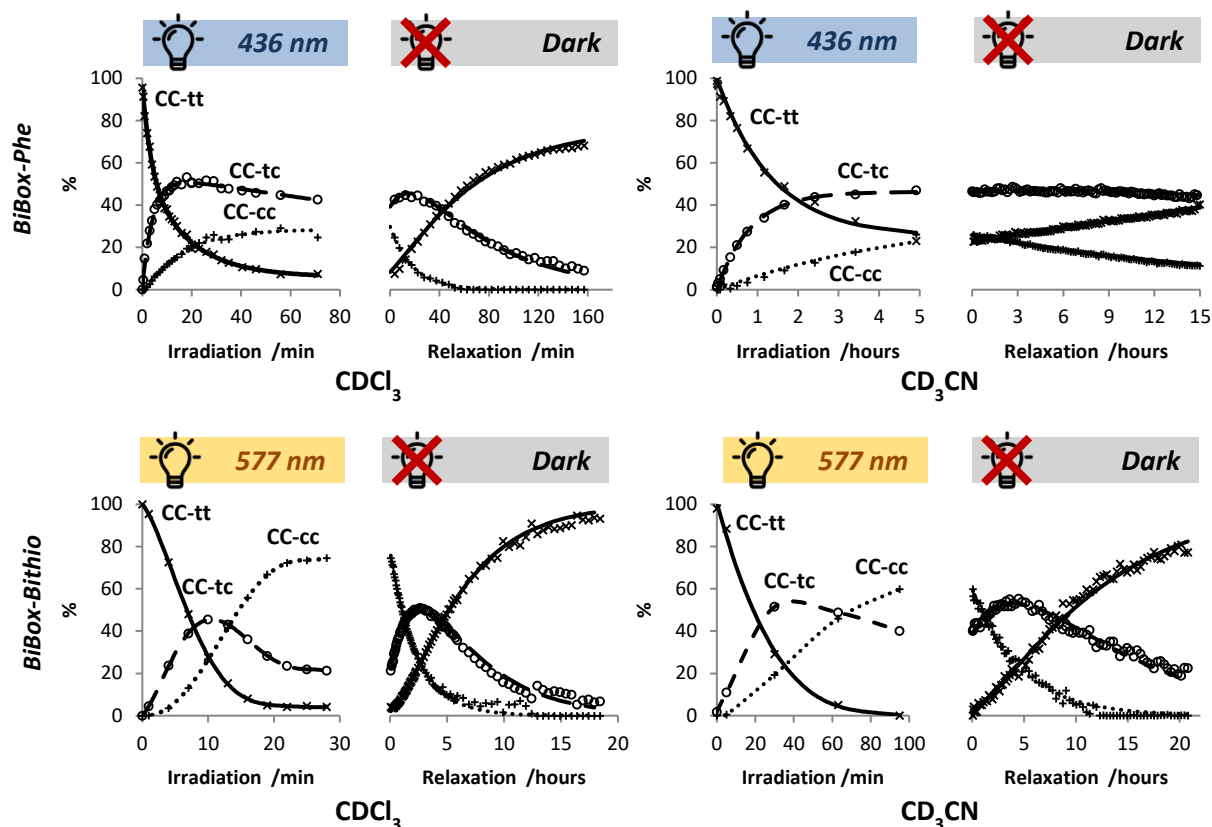


Figure 6. Kinetics of the photoisomerization of CC-tt state of BiBox-Phe (-Bithio) upon 436 nm (577 nm) light irradiation and thermal relaxation.

Effect of visible light irradiation on OC-tt state. (Scheme 2, column b)

The OO-tt and CC-tt states showing a photoswitchable behavior, the photoreactivity of the dissymmetrical state OC-tt was investigated upon irradiation with 436 nm and 577 nm light for BiBox-Phe and -Bithio, respectively. As shown previously, upon 1.5 equivalents of DCl, the maximal OC-tt concentration could be reached in acetonitrile. Experiments were only done in acetonitrile as aqueous hydrochloric acid was miscible with chloroform. was applied.

As samples were constituted of a mixture of CC, OC and OO species in trans-trans configuration, several conversions were observed and among them, the previously described transformations of CC-tt and OO-tt. Irradiation at 436 nm allowed to convert 50% of OC-tt state of BiBox-Phe into a new state characterized by doublets at 6.83 and 5.89 ppm ($J = 13.2$ Hz), and doublets at 8.33 ppm and 7.69 ppm ($J = 16.3$ Hz). These chemical shifts imply that the cis double bond is connected to the closed Box moiety and the trans double bond is connected to the open Box moiety, thus the state is identified to OC-tc (Figure S7 a). Neither the OC-ct nor OC-cc states were detected. OC-tc turned so rapidly to OC-tt that its rate constant could be measured. For BiBox-Bithio, irradiation with a 577 nm light led to convert the OC-tt state into

65% of OC-tc and 35% of OC-cc. OC-tc was identified by two doublets at 8.35 and 7.19 ppm ($J = 16.1$ Hz) for the trans protons aside from the open Box moiety, and two doublets at 6.82 and 5.60 ppm ($J = 13.1$ Hz) for the cis ethylenic protons near the closed Box. As for OC-cc, two pairs of doublets for ethylenic protons showed a 13.0 and 12.8 Hz cis coupling constant for both double bonds (Figure S7 b). No other state was detected in our experimental conditions. Finally, the kinetic of the thermal back reaction in the dark was recorded and the concentrations of OC-tt, OC-tc and OC-cc were plotted against time (Figure 7). While OC-cc totally disappeared after less than 20 minutes, the OC-tc lifetime was found much longer, as 10 hours were necessary to recover the initial OC-tt concentration. Fitting the experimental data using a kinetic model for two consecutive reactions showed the first thermal back isomerization to be 60 times faster than the second one (Table 1. c). The very short lifetime of OC-cc indicated how less stable a cis ethylenic junction is when directly connected to an open Box moiety compared to a closed one. This is explained by the lower electronic density around the ethylenic junction next to the open Box due to withdrawing effect of the iminium moiety. This observation also explains why OC-ct is not detected, as the cis isomery next to the open box is not stable compared to the other way round

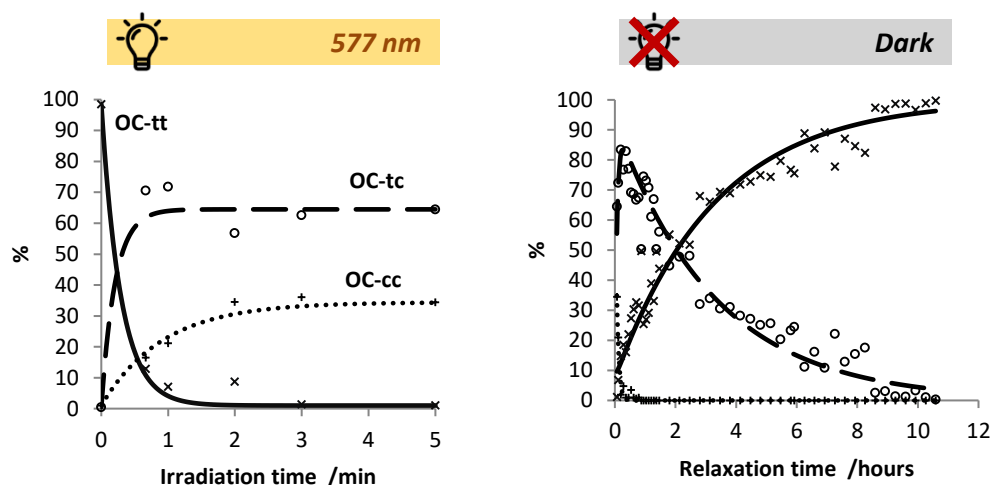


Figure 7. Evolution of OC-tt, OC-tc and OC-cc normalized concentrations for BiBox-Bithio (left) under 577 nm irradiation and right) the thermal back reaction in the dark.

Effect of acid on the Box opening in the CC-tc and CC-cc states (Scheme 2, rows b and c)

At this point, generation of cis (-tc and -cc) isomers by photoirradiation has been extensively studied, whatever the closed or open states of the BiBox systems. We now address the question whether opening-closing of Box moieties is possible for those cis isomers, allowing cross conversions between all nine species defined so far. The two CC-tc and CC-cc states of BiBox-Bithio were accumulated upon irradiation at 577 nm of CC-tt in acetonitrile and then 1.5 eq. of hydrochloric acid was added (Figure S8). NMR spectroscopy revealed only the appearance of the OC-tc state with characteristic signals as described previously. Unfortunately, the OC-cc state resulting from acidification of CC-cc was not observed in our experimental conditions probably due to its short lifetime. Interestingly, the addition of an excess of trifluoroacetic acid vapor on a mixture of CC-tc and CC-cc in chloroform allowed an instantaneous conversion into the OO-tc and OO-cc, respectively (Figure S9). Finally, upon an excess of base (triethylamine vapor), the quantitative closing of OO-tc and OO-cc towards CC-tc and CC-cc, respectively was achieved. Thus, the acid opening and the base closing of the Box unit could be induced whatever the trans or cis configuration of the ethylenic junction.

CONCLUSION

Based on the literature, BiBox systems have already shown promising switching features, by solely taking into account the commutation of the Box moiety.²⁴ So far, the present work stood for a proof of concept of extending the accessible states of such molecular systems by taking advantage of the switching ability of ethylenic junctions upon specific irradiation. Nine commutation states were

characterized for the two BiBox systems and the pathways to switch from one to another were clearly identified by playing with light, acid, oxidant, base and temperature. No degradation was observed proving its robustness. In addition, the modulation of emission properties depending on the closed or open state of the Box moieties and the effect of the aromatic bridge on these properties was also highlighted. Especially, the opening of one Box moiety induced a decrease in the fluorescence quantum yield accompanied with a shift towards longer wavelengths of both absorption and emission bands, except for BiBox-Phe for which the emission bands remained in the same region. The opening of the second Box allowed for recovering an intermediate fluorescence quantum yield and the emission band shift remained discrete.

To conclude, the present work evidenced the promising multiaddressable and multiresponsive properties of molecular systems having four switchable units, based on their commutation and emission properties, and thus opens new perspectives for Box-based molecular switches.

MATERIALS AND METHODS

Analytical methods – NMR, UV-Vis and fluorescence spectroscopies

NMR spectra were recorded on Bruker NEO ¹H 500 MHz - equipped with a TXI probe using standard pulse sequences. Samples in acetonitrile-*d*₃ and chloroform-*d*₂ were prepared in glass 5 mm NMR tubes. Spectra were processed with Bruker Topspin 4.0.2. UV-Visible spectra were acquired with a Varian Cary 50 single beam spectrometer with a 0.1 s integration time every 1 nm. Solvents used are commercially available spectroscopic grade acetonitrile and chloroform without further purification. Quartz cuvettes were used with a 1 cm optical path. Typical concen-

trations for NMR spectroscopy were 1.0 to 3.0.10⁻⁴ M and 0.8 to 2.0.10⁻⁵ M for UV-Vis.

Emission spectra were acquired with a Jobin Yvon Fluoromax 3 spectrofluorometer with 5 nm excitation and emission slits width and integration time was set to 0.1 s. A Varian Cary 100 UV-Visible spectrophotometer was used to obtain absorption spectra before and after fluorescence spectra in order to monitor possible photoreaction after excitation.

Irradiation setup

NMR samples were directly irradiated inside rotating 5 mm NMR tubes on a custom temperature regulated bench, with a Newport 1000 W high pressure Hg-Xe lamp filtered to desired wavelength and focused on a quartz light guide to the sample. *In situ* irradiation setup was used for UV-Vis spectroscopy, with a filtered Oriel 100 W high pressure Hg lamp, fibred to the sample.

Filters used to select irradiation wavelength are interference or high pass filters to follow photo reaction both by NMR and UV-Vis spectroscopy. Irradiation at 436, 546 and 577 nm were carried out using interference filters (Andover Corporation) with maximum transmittance of 16, 17 and 19% at respective wavelengths at 434, 536 and 582 nm, with a 10 nm FWHM. For irradiation at $\lambda > 495$ nm, a high pass GG495 filter was used. Those filters were coupled with a KG1 heat absorbing filter.

Acid and oxidant titration

Acid titrant solutions were prepared from stock DCl 35 wt. % in D₂O (99% D) commercial solution, diluted in acetonitrile-d₃ (99,8% D), while known mass of solid nitrosonium hexafluoroantimonate (NOSbF₆) was dissolved in the same solvent (acetonitrile-d₃) to obtain oxidant titrant solution. Concentration of each titrant solution was adjusted so that 50 μ L of it contained 1.0 equivalent of the 500 μ L BiBox titrated solution. The same procedure was used when the titration was followed by UV-Visible and fluorescence spectroscopy, but with non-deuterated solvents (acetonitrile) and acid (HCl), and the initial volume of the BiBox titrated solutions was 3 mL.

Compounds and references solutions for fluorescence spectroscopy

Studied compounds and references were dissolved in spectroscopic grade solvents without further purification (acetonitrile VWR Spectronorm, ethanol Uvasol Merck). Solutions were prepared directly into the 1.0 cm optical path spectroscopic cell and concentration was adjusted so that absorbance at the excitation wavelength was below 0.1 to avoid inner filter effect. Typical concentrations were of μ M order of magnitude. Hydrochloric acid solution used to promote opening of the oxazolidine moieties was obtained from 35% w/w aqueous hydrochloric acid solution diluted in acetonitrile.

Fluorescence data treatment

Solvent contribution to fluorescence spectra was subtracted, allowing excitation harmonic removal as well. Fluorescence quantum yields ($\Phi_C^{\lambda_{exc}}$) were obtained relatively to well-documented reference compound quantum yields ($\Phi_R^{\lambda_{exc}}$). These reference compounds were chosen in order to match the absorption and emission bands with the investigated compound. Reference quantum yields and corresponding experimental conditions (solvent, temperature) were found from Brouwer's 2011 report²⁴ and corresponding original papers gathered in Table 2.

Table 2. Reference compounds and conditions used to determine fluorescence quantum yields of Box compounds

Name	Excitation	Emission	ϕ_R	Solvent	Ref
Phenanthrene	260-335	345-410	0.125	EtOH	²⁶
Quinine Sulfate	280-380	380-580	0.546	H ₂ SO ₄ 0.5M	^{26,27}
Coumarine 153	370-470	480-650	0.38	EtOH	²⁸
Rhodamine 101	500-550	600-650	1.0	MeOH	²⁹

Absorption at excitation wavelength ($A^{\lambda_{exc}}$) and integration of emission spectra (I) of both analyzed (C) and reference (R) compounds as well as refractive index (n) of each solvents were necessary to obtain the fluorescence quantum yield of any compound ($\Phi_C^{\lambda_{exc}}$) from formula (1).

$$\Phi_C^{\lambda_{exc}} = \Phi_R^{\lambda_{exc}} \times \frac{(1 - 10^{-A_R^{\lambda_{exc}}}) \times I_C \times n_C^2}{(1 - 10^{-A_C^{\lambda_{exc}}}) \times I_R \times n_R^2} \quad (1)$$

ASSOCIATED CONTENT

Supporting Information. ¹H NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

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