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# Synthesis and Multistate Characterization of *bis*-Flavylium Dications – Symmetric Resorcinol- and Phloroglucinol-Type Derivatives as Stochastic Systems

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Two symmetric *bis*-flavylium dications were readily synthesized and further evaluated for their multistate profile. Both systems were shown fully stochastic and behave like a single compound suggesting that the two flavylium moieties do not communicate *via* the bridge linking them. Global  $pK_a$  values of *ca.* 4 regarding the acid-base reaction between flavylium cation and quinoidal base were calculated by stopped flow. It was further demonstrated that the equilibrium between  $AH^*-AH^*$  and indistinguishable flavylium-quinoidal base isomers  $AH^*-A$  ( $A-AH^*$ ) can be calculated by subtracting 0.3 pH units to the observed acid-base constant. On the other hand, the equilibrium between both flavylium-quinoidal base **a**-**A** is obtained by adding 0.3 pH units. Moreover, both systems do not have *cis-trans* isomerization barrier and the rate constants of interconversion between flavylium cation and quinoidal base as a function of pH are fitted with a mono-exponential and follow a bell-shaped curve. The systems proved also to be photochromic and from the fitting of the bell-shaped curve, flash photolysis measurements and quantum yields, it is possible to calculate all rate and equilibrium constants and construct a global energy-level diagrams. It was also proved that the rate constant to form both isomers  $AH^+-C_t$  from the *bis*-flavylium upon a pH jump from 1 to less acidic solutions is twice of the observed value and the formation of the *bis*-trans-chalcone from the both isomers  $AH^+-C_t$  is equal to the observed rate constant. An energy-level diagram of all the multistate species was constructed from the equilibrium constants.

# Introduction

Flavylium (2-phenyl-1-benzopyrylium) derivatives constitute an important family of natural pigments, which comprise *i*) anthocyanins, the molecules responsible for most of the red and blue colors of flowers and fruits,<sup>1,2</sup> and *ii*) 3-deoxyanthocyanidins, the colorants appearing in ferns and mosses as well as hybrids of sorghum and purple corn<sup>3</sup>. Besides the above mentioned natural pigments, a myriad of non-natural flavylium derivatives have been synthesized, some of them exhibiting relevant photochromic properties with practical applications as models for optical memories, molecular logic gates and even to mimic elementary properties of neurons.<sup>4</sup>

From a structural viewpoint, flavylium compounds are generally defined by the respective flavylium cation, which is nevertheless only one of the species of a reversible sequence of chemical reactions that are interconnected by external stimuli, such as pH, light and removing or adding electrons (redox).<sup>4b,5</sup> As illustrative example, if one considers oenin which is the major flavylium-based

pigment of European grapevine species (*ie*, Vitis vinifera), this natural anthocyanin is known to be pushed into a well-established multiequilibra network involving five distinct structural forms: the red-colored flavylium cation ( $AH^+$ ) and its colored quinoidal conjugate base (A), the colorless hemiketal species (B) as well as its colorless (or pale-yellow) ring-opened counterparts ( $C_c$  and  $C_t$ ) (Scheme 1).<sup>6</sup>

In order to characterize such a flavylium multistate system,<sup>7,8</sup> it is necessary to perform direct pH jumps defined as the addition of base to equilibrated solutions of the flavylium cation (generally for pH<1), thus triggering the sequence of reactions shown in Scheme 1. Also it is required to carry out reverse pH jumps, obtained by addition of acid to equilibrated solutions at higher pH values.

Thus, after a direct pH jump, the flavylium cation  $(AH^{+})$  yields the quinoidal base (A) upon proton transfer in competition with hydration in position 2 to give the hemiketal (B) (Figure 1, Equ.(a) vs Equ.(b)). The proton transfer is by far the faster reaction of the multistate and by consequence A is formed before B. One important breakthrough in this system was reported in the late seventies by Brouillard and Dubois, who showed that A does not hydrate in acidic medium.<sup>9</sup> Consequently, the evolution of the system takes place through the hemiketal while the quinoidal base is a kinetic product that is in fast equilibrium and disappears simultaneously with  $AH^{+}$ . On the other hand, hemiketal B could further open ring C and form *cis*-chalcone (C<sub>c</sub>) (Figure 1, Equ.(c)), itself in slow isomerization equilibrium with the *trans*-chalcone (C<sub>c</sub>)

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(Figure 1, Equ.(d)). At higher pH values, ionized species are formed by deprotonation of the remaining hydroxyl groups (not shown in Scheme 1). Altogether, the system can be simplified considering a single acid base equilibrium involving the species  $AH^{+}$  (acid) and **CB** (basic) where **CB=A+B+C<sub>c</sub>+C<sub>t</sub>** (Figure 1, Equ.(e)).



Scheme 1. Flavylium multistate systems – the case of oenin as illustrative example.

$\mathbf{AH^{+}} + \mathbf{H}_{2}\mathbf{O} \xrightarrow{k_{a}} \mathbf{A} + \mathbf{H}_{3}\mathbf{O}^{+}$	proton transfer K <sub>a</sub>	Equ.(a)	
$\mathbf{AH^{+}} + 2H_2O \underbrace{k_h}_{k_{-h}} \mathbf{B} + H_3O^+$	hydration K <sub>h</sub>	Equ.(b)	
$\mathbf{B}  \stackrel{k_t}{\underbrace{k_t}}  \mathbf{C}_{\mathbf{c}}$	ring-opening K <sub>t</sub>	Equ.(c)	
$\mathbf{C}_{\mathbf{c}} \xrightarrow{k_i} \mathbf{C}_{\mathbf{t}}$	isomerization	Equ.(d)	
$\mathbf{AH^{+} + H_{2}O} \xrightarrow{k_{a}} \mathbf{CB} + H_{3}O^{+}$	acid-base global equilibrium K' <sub>a</sub>	Equ.(e)	
with $\mathbf{K}_n = \frac{k_n}{k_n}$ $(n = a, h, t, i)$ and $\mathbf{K}_a = \mathbf{K}_a + \mathbf{K}_h + \mathbf{K}_h \mathbf{K}_t + \mathbf{K}_h \mathbf{K}_t \mathbf{K}_i$			

**Figure 1.** Flavylium multistate systems – summary of the implicated chemical equilibria and the corresponding kinetic/thermodynamic constants.

Regarding the kinetic processes after a direct pH jump, the respective mathematical expressions are different according to the existence or the lack of the *cis-trans* isomerization barrier. In the first case, as in anthocyanins, three distinct steps are observed (Figure 2, Math.(a-c)). The first corresponds to the proton transfer, the second to the hydration, considering that tautomerization is faster (which is the general case except for very acidic pH values in reverse pH jumps experiments), and the third is the isomerization reaction.

Regarding the reverse pH jumps carried out from equilibrated or pseudo-equilibrated solutions, equations (d) and (e) presented in Figure 2 account for the respective kinetics. The first one is observed when the final pH of the reverse pH jump is less acidic, while the second corresponds to the case of regime change: the hydration becomes faster than tautomerization at very high proton concentrations, and the last reaction is the controlling step of the kinetic process. In the absence of the *cis-trans* isomerization barrier only one kinetic process (besides proton transfer) is observed as reported in equation (f) (Figure 2). Representation of this rate constant (*ie*,  $k_{obs}$ ) as a function of pH is a bell shaped curve, where the left branch is controlled by the isomerization reaction while the right branch by the hydration.<sup>10</sup>

$$k_{i} = k_{a} + k_{a} \left[ H^{+} \right] \qquad \text{(Math.(a))}$$

$$k_{2} = \frac{\left[ H^{+} \right]}{\left[ H^{+} \right] + \mathbf{K}_{a}} k_{h} + \frac{1}{1 + \mathbf{K}_{t}} k_{h} \left[ H^{+} \right] \qquad \text{(Math.(b))}$$

$$k_{3} = \frac{\mathbf{K}_{h} \mathbf{K}_{t}}{\left[ H^{+} \right] + \mathbf{K}_{a} + \mathbf{K}_{h} + \mathbf{K}_{h} \mathbf{K}_{t}} k_{i} + k_{.i} \qquad \text{(Math.(c))}$$

$$k_{1st \ reverse} = \frac{\left[ H^{+} \right]}{\left[ H^{+} \right] + \mathbf{K}_{s}} k_{h} + k_{.h} \left[ H^{+} \right] \qquad \text{(Math.(d))}$$

$$f_{ist reverse} = \frac{1}{[H^+] + K_a} k_h + k_{-h} [H^+] \qquad (Math.(d))$$

$$k_{2nd reverse} = k_{-t} + k_{-t}^{H} [H^{+}] + k_{-t}^{OH} [OH^{-}]$$
 (Math.(e)

$$k_{obs} = \frac{\frac{|H^{+}|}{|H^{+}| + K_{a}} K_{h}K_{t}k_{i} + k_{i} |H^{+}|}{\frac{K_{t}k_{i}}{k_{i}} + |H^{+}|} \qquad (Math.(f))$$

Figure 2. Flavylium multistate systems – mathematical expressions of the key kinetic constants.

To go further, shifting from flavylium monocations to more sophisticated dicationic flavylium-based skeletons appears attractive, because enabling the network of chemical reactions to be influenced/extended by the presence of the second cationic moiety. Although the synthesis of such sophisticated flavylium-based systems, as flavylium-benzopyrylium<sup>11</sup> skeleton **A** and *bis*-flavylium skeletons **B**- $E^{12}$ , has been reported in the literature (Figure 3), their multistate characterization remains surprisingly very few documented. To our knowledge, the *bis*-flavylium **C** where two flavylium units are linked *via* a redox-responsive methyl viologen bridge constitutes the only system whose multistate behavior has been evaluated to date.<sup>12b</sup> Remarkably, the performed studies have revealed that the system is not fully stochastic, especially regarding the isomerization event (Figure 1, Equ.(d)).

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In this context and due to our interest in flavylium chemistry<sup>4b,11,12a,13</sup> as well as their potent applications<sup>4b-c,8,14</sup>, we report herein on the synthesis and the full multistate characterization of two symmetric *bis*-flavylium dications, the resorcinol- and phloroglucinol-type salts **1** and **2** respectively (Figure 4).



**Figure 3.** Selected examples of flavylium-based dications **A-D** and tetracation **E** (<sup>*a*</sup>See ref. 11; <sup>*b*</sup>See ref. 12a; <sup>*c*</sup>See ref. 12b; <sup>*d*</sup>See ref. 12c; <sup>*c*</sup>See ref. 12d).



Figure 4. Symmetric  $\mathit{bis}$ -flavylium dications 1 and 2 studied in the present work.

# **Results and discussion**

#### Synthesis

The synthesis of bis-flavylium 1 and 2 was achieved in line with a procedure recently reported by us (Scheme 2).<sup>12a</sup> The procedure consists of a four-step sequence taking advantage of the acid-mediated condensation between bisarylethynylketone 5 and resorcinol/phloroglucinol as last and key step. The required condensation partner 5 was first prepared in three steps starting from commercially available and cheap materials, namely 4-hydroxybenzaldehyde and 1,3dibromopropane. After coupling two equivalents of 4hydroxybenzaldehyde with one equivalent of the dibrominated alkylating agent, the resulting bis-benzaldehyde 3 was then efficiently converted to 5 via a nucleophilic addition-oxidation sequence, involving bis-propargynol derivative 4 as intermediate and employing ethynyl magnesium bromide as nucleophilic reagent and 2iodoxybenzoic acid (IBX) as oxidant. With 5 in hand, the key condensation step proved highly efficient with either resorcinol or phloroglucinol, furnishing the desired *bis*-flavylium **1** and **2** in almost quantitative yields. The easy-toperform 'click' feature of the *bis*-condensation deserves here to be reminded, notably because the dications **1**/**2** are isolated in pure forms due to a very simple work-up (*ie.* a filtration/washing sequence). Also worth noting is that both dications were obtained with an excellent efficiency over the four steps, *ie.* 75% and 74% overall yields for **1** and **2** respectively.



Scheme 2. Synthesis of bis-flavylium dications 1/2. Reagents and conditions: a) 1,3-dibromopropane (1.0 equiv.), 4-hydroxybenzaldehyde (2.4 equiv.),  $K_2CO_3$  (3.0 equiv.), DMF, 100 °C, 12 h; b) 3 (1.0 equiv.), HCCMgBr (2.6 equiv.), THF, 0°C to rt, 3 h; c) 4 (1.0 equiv.), IBX (4.0 equiv.), EtOAc, 80 °C, 12 h; d) 5 (1.0 equiv.), resorcinol (R=H) or phloroglucinol (R=OH) (2.0 equiv.), aqueous HPF<sub>6</sub> (xs), AcOH, rt, 48 h.

#### Characterization of multistate systems

With these two symmetric *bis*-flavylium 1/2 in hand, we next investigated their multistate behavior, taking into account as starting hypothesis that two equal flavylium multistates are present in the molecules.

#### Physicochemistry

The pH dependent spectral variations of compounds **1** and **2** monitored by stopped flow 10 ms after a direct pH jump are shown in Figure 5A and Figure 5B respectively. Inspection of these figures indicates that these spectral changes are compatible with a single acid-base equilibrium involving the cationic flavylium forms and its quinoidal bases. The isosbestic points show that flavylium forms disappear by increasing pH to give exclusively the quinoidal bases (in this time scale). In the

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cases of both compounds **1/2**, fitting was achieved by a single acid-base equilibrium with  $pK_a(1)=3.9$  and  $pK_a(2)=4.0$ .



**Figure 5.** UV/Vis spectral variations occurring upon a direct pH jump from stock solutions of compounds **1/2** at pH1 ( $2.5 \times 10^{-5}$  M/9.3 $\times 10^{-6}$  M) to higher pH values. Spectra recorded immediately after the pH jump in the case of **1 (A)** and in the case of **2 (B)**. Spectra recorded at the thermodynamic equilibrium in the case of **1 (C)** and in the case of **2 (D)**. Insets: fittings (–) of the absorbance values ( $\bullet$ / $\bigcirc$ ) at the specified wavelengths.

The global acid-base equilibrium can be accounted for by equation Equ.(f) and the network of chemical reactions depicted in Scheme 3.<sup>8,15</sup> The  $pK_{a1}$  is defined as the acid-base equilibrium between the diprotonated form  $(A_2H_2^{2^+})$  and the sum of the monoprotonated species  $(A_2H^+)$ , while  $pK_{a2}$  between the monoprotonated species  $(A_2H^+)$  and the neutral form  $(A_2)$ . As mentioned above, both systems behave as a single acid-base equilibrium corresponding *i*) in the case of 1, to the stochastic constant  $pK_{a1}(1)=3.9$  and by consequence  $pK_{a1}(1)=3.6$  and  $pK_{a2}(1)=4.2$ , and *ii*) in the case of 2, to  $pK_{am}(2)=4.0$ ,  $pK_{a1}(2)=3.7$  and  $pK_{a2}(2)=4.3$ . Accordingly, these data then allowed us to determine the mole fraction distribution of the respective species as shown in Figures 6A-B.



Scheme 3. Deprotonation sequences of the bis-flavylium.<sup>1</sup>



**Figure 6.** Mole fraction distribution of the diprotonated-monoprotonated and neutral species of the equilibrium *i*) between flavylium cation and quinoidal base in the case of **1** (**A**) and in the case of **2** (**B**), and *ii*) at the thermodynamic equilibrium in the case of **1** (**C**) and in the case of **2** (**D**).

Remarkably, both *bis*-flavylium systems also behave as a single acidbase at the thermodynamic equilibrium, thus involving flavylium cation and *trans*-chalcone (Figure 1, Equ.(e)). Figures 5C-D indeed show the pH-dependent absorption of solutions of **1/2** after equilibration for *ca*. 24h, revealing an extremely small  $pK'_a$  value for 1 (*ie*. 0.7 and 3.3 for **1** and **2** respectively). These values compare with  $pK'_a=1.5$  for the model compound 4'-methoxy-7hydroxyflavylium in the same conditions (*ie*. 50% water/ethanol, v/v, room temperature, see Figure S1 in SI). Considering again the systems as stochastic, the corresponding global equilibrium can be accounted for by equation Equ.(g) depicted in Figure 7. The same reasoning as above then leads to  $pK'_{a1}(1)=0.4$  and  $pK'_{a2}(1)=1.0$ (compares with  $pK'_{a1}(1)=3.0$  and  $pK'_{a2}(1)=3.6$  for **2**), further confirming the unusual higher acidity of the *bis*-flavylium **1**.

$$H^{+} - AH^{+} \xrightarrow{K'_{a1}} AH^{+} - C_{t} \xrightarrow{K'_{a2}} C_{t} - C_{t} \quad \boxed{\text{Equ.(g)}}$$
(where  $AH^{+} - C_{t}$  accounts for both isomers)

Figure 7. Deprotonation sequence of the *bis*-flavylium at the thermodynamic equilibrium.

Having proved that the systems **1/2** are both stochastic, the following discussion considers that similarly to the acid-base reaction all micro-rate and equilibrium constants are the same and the systems can be treated as a single one multistate flavylium.

From the spectra of Figures 5A-B to the spectra at the equilibrium (Figures 5C-D), the systems evolve according to a single monoexponential decay as clearly shown in the insets of Figures 8A-B. First, regarding the *bis*-flavylium **1**, representation of a series of direct pH jumps as a function of pH was fitted with Equ.(f) (Figure 2) for the parameters  $pK_a(\mathbf{1})=3.9$ ,  $K_hK_tk_i=1.15\times10^{-6}$  M<sup>-1</sup>s<sup>-1</sup>,  $k_{\cdot i}=10^{-5}$  s<sup>-1</sup> and  $K_tk_i/k_{\cdot h}=1.9\times10^{-4}$  M<sup>-1</sup>s<sup>-1</sup> (Figure 8C). At extremely low pH values, a term equal to  $k_{cat}^{H}$  [H<sup>+</sup>] should be added,  $k_{cat}^{H}=7.6\times10^{-4}$  M<sup>-1</sup>s<sup>-1</sup> should be added to account for a catalytic step recently observed as a general behavior of flavylium networks most probably due to the Published on 22 June 2016. Downloaded by test 3 on 26/06/2016 15:16:20

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catalytic effect by proton on the chalcones isomerization. Of note the so-obtained representation of the  $k_{obs}$  rate constant as a function of pH is a typical bell-shaped curve, where the left branch is controlled by the isomerization reaction while the right branch by the hydration.



**Figure 8.** (A) Spectral variations of **1** after a direct pH jump from pH 1 to pH 5 -Inset: fitting of the absorbance at 493 nm gives  $k_{obs} = 3.5 \times 10^{-4} \text{ s}^{-1}$  at pH 3.9. (B) Spectral variations of **2** after a direct pH jump from pH 1 to pH 6.3 – Inset: fitting of the absorbance at 493 nm gives  $k_{obs} = 7.0 \times 10^{-5} \text{ s}^{-1}$  at pH 4. (C) Regarding **1**, representation of a series of direct pH jumps ( $\bullet$ ) and reverse pH jumps (O) from pH 7. (D) Same as C but in the case of **2**. (E) Decay of the flavylium cation **1** and *trans*-chalcone formation after a direct pH jump as in the inset of (A) (red lines) - kinetics of the *bis*-flavylium disappearance to give the intermediate with a unique flavylium and the final *bis*-trans-chalcone.

In a similar way, fitting of the experimental data in Figure 8B with Equ.(g) (Figure 2) was achieved for the parameters  $pK_a(2)=4.0$ ,  $K_hK_tk_i=1.3x10^{-8} \text{ M}^{-1}\text{s}^{-1}$ ,  $k_{\cdot i}=2.2x10^{-5} \text{ s}^{-1}$ ,  $K_tk_i/k_{\cdot h}=8.5x10^{-7} \text{ M}^{-1}\text{s}^{-1}$  and  $k_{cat}^{H}=1.5x10^{-3} \text{ M}^{-1}\text{s}^{-1}$  (Figure 8D).

As shown in Appendix 2 given in SI and taking into account that the systems are stochastic, the rate constants for the formation of the species  $AH^+-AH^+$ ,  $AH^+-C_t$  ( $C_t-AH^+$ ) and  $C_t-C_t$  can be calculated. Interestingly enough, it can be proved that the intermediate species bearing one flavylium cation and a *trans*-chalcone at each terminal is formed with a rate constant two times higher than the rate

constant of the observed global process and disappears with the same rate constant of the global process.

#### Photochemistry

In the case of flavylium multistates lacking the *cis-trans* isomerization barrier the number of relations between rate and equilibrium constants of the system is not enough to carry out their full characterization. However, in some cases it is possible to use other stimuli rather than pH jumps to shift the multistate from the equilibrium and follow the relaxation processes and in this way get more information regarding the multistate. This is the case of the *trans-cis* photo-induced  $C_t=C_c$  isomerization.

As shown in Figures 9A-B, the present *bis*-flavylium compounds 1/2 both exhibit photochromism because upon irradiation, the absorption band of the flavylium cation increases at the expenses of its *trans*-chalcone form. Furthermore, representation of the quantum yields as a function of pH are presented in Figures 9C-D.



**Figure 9.** (A) Spectral variations upon irradiation of 1 at pH=3.8,  $\mathbb{D}_{irr}$  =376 nm. (B) Spectral variations upon irradiation of 2 at pH=4.2,  $\mathbb{D}_{irr}$ =370 nm. (C) Quantum yields of the photochemical reaction involving 1 as a function of pH - Fitting was achieved with Math.(k) and (I) (Figure 11). (D) Same as C but in the case of 2.

For clarity reasons, the following discussion will focus more particularly on *bis*-flavylium **1**. The flash photolysis of **1** was carried at different pH values, and is shown for equilibrated solutions at pH 2 (Figure 10A). After the flash, there is a bleaching at 376 nm, a wavelength where *trans*-chalcone  $C_t$  shows its absorption maximum. The bleaching occurs because the species formed is *cis*-chalcone  $C_c$  that has a low molar absorption coefficient at this wavelength. The absorption at 376 nm is partially recovered in *ca*. 0.2 s at this pH value, indicating that part of *cis*-chalcone  $C_c$  gives back *trans*-chalcone  $C_t$ . At the same time and with identical lifetime, the flavylium cation is formed and as a result, the fraction of non-recovered *trans*-chalcone  $C_t$  corresponds to the fraction of so-

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formed flavylium cation. Representation of the rate constants of the flash photolysis experiments is shown in Figure 10A and, as observed for other flavylium multistates, the rates of the flash photolysis follow two regimes. At higher pH values, since the hydration is much slower than tautomerization, the equilibrium between hemiketal and *cis*-chalcone is attained and Math.(g) (Figure 11) could be used. At lower pH values occurs the change of regime and hydration is much faster than tautomerization. Thus, all the hemiketal that is formed upon ring closure gives immediately flavylium cation and Math.(h) (Figure 11) should be used for fitting.



**Figure 10.** (A) Traces of the flash photolysis of 1 at pH 2. (B) Rate constants of the flash photolysis as a function of pH - Fitting was achieved with Math.(g) and Math.(h) (Figure 11).

$$k_{flash(hydration)} = \frac{|H^+|}{|H^+| + \mathbf{K}_a} k_h + \frac{1}{1 + \mathbf{K}_t} k_{-h} [H^+] + \frac{\mathbf{K}_t}{1 + \mathbf{K}_t} k_i \quad (Math.(g))$$

$$k_{\text{flash}(\text{tautomerization})} = k_i + k_{-t} + k_{-t}^H [H^+] + k_{-t}^{OH} [OH^-]$$

$$k_{backward} = \frac{\mathbf{K}_t}{1 + \mathbf{K}_t} \quad k_i$$
 (Math.(j))

$$\Phi = \Phi_{o} \frac{k_{forward}}{k_{forward} + k_{backward}} = \frac{|H^{+}|}{\frac{\mathbf{K}_{l}k_{i}}{\mathbf{K}_{\cdot h}} + |H^{+}|}$$
(Math.(k))

$$\Phi = \Phi_{0} \frac{k_{t} + k_{t}^{H} [H^{+}] + k_{t}^{OH} [OH^{-}]}{k_{t} + k_{t} + k_{t}^{H} [H^{+}] + k_{t}^{OH} [OH^{-}]}$$
(Math.(I))

**Figure 11.** Flavylium multistate systems – mathematical expressions of the kinetic constants and quantum yields related to the photoinduced isomerization process.

The pH-dependent quantum yields reported in Figure 9C provide also significant kinetic information that can be used to calculate the rate and equilibrium constants of the system. When the hydration is the rate-determining step, the equilibrium between B and C<sub>c</sub> is established and the forward and backward reactions are respectively given by Math.(i) (Figure 11) and Equ.(e) (Figure 1), assuming that the tautomerization equilibrium is achieved.<sup>16</sup> Further neglecting the contribution of  $k_{i}$  as well as  $k_h[\text{H}^+]/([\text{H}^+]+K_a)$ ,<sup>17</sup> the pH-dependence of the quantum yield is thus given by Math.(k) (Figure 11).

On the other hand, when the rate determining step is the tautomerization reaction as observed in Figure 10, the pH dependence of the quantum yield then follows Math.(I) (Figure 11). A global fitting was finally carried out using Equ.(e) and Math.(f-I), the resulting values of the parameters being summarized in Table 1.

	Table 1. Fitting parameters used to fit kinetic data				
	<b>Κ</b> <sub>h</sub> <b>Κ</b> <sub>t</sub> <b>k</b> <sub>i</sub> (M <sup>-1</sup> s <sup>-1</sup> )	<b>K<sub>t</sub>ki/k₋</b> <sub>h</sub> (M <sup>-1</sup> s <sup>-1</sup> )	<i>k.<sub>h</sub>/</i> (1+K <sub>t</sub> ) (M <sup>-1</sup> s <sup>-1</sup> )	<b>k</b> <sub>i</sub> <b>K</b> <sub>t</sub> / (1+K <sub>t</sub> ) (s <sup>-1</sup> )	<b>k</b> <sub>i</sub> + <b>k</b> <sub>-t</sub> (s <sup>-1</sup> )
1	1.5x10 <sup>-6</sup>	1.9x10 <sup>-4</sup>	1.4x10 <sup>3</sup>	0.26	0.36
2	1.3 x10 <sup>-8</sup>	8.5x10 <sup>-7</sup>	6.0x10 <sup>4</sup>	0.03	0.7

With  $k^{H} = 5 \text{ M}^{-1}\text{s}^{-1}$  and  $k^{OH} = 2x10^{9} \text{ M}^{-1}\text{s}^{-1}$  for **1** and  $k^{H} = 200 \text{ M}^{-1}\text{s}^{-1}$ and  $k^{OH} = 5x10^{9} \text{ M}^{-1}\text{s}^{-1}$  for **2**.

#### Full characterization

From the parameters reported in Table 1, it is possible to calculate all the rate and equilibrium constants of the system 1 and thus fully characterize its multistate behavior. The data of the rate and equilibrium and kinetic constants of 1 are presented respectively in Table 2 and Table 3. In parallel, the full multistate characterization of *bis*-flavylium 2 was performed using the same overall reasoning and the calculated values are also given in Table 2 and Table 3.

Math.(h)					
	Table 2. Equilibrium constants of bis-flavylium 1 and 2.				
Math.(i)		р <i>К</i> а / р <i>К</i> 'а	$\boldsymbol{K}_{h}(M^{-1})$	K	Ki
Math (i)	1	3.9 / 0.9 <sup>[a]</sup>	1.2x10 <sup>-6</sup>	2.7	3.55x10 <sup>4</sup>
	2	4.0 / 3.3	3.5x10 <sup>-7</sup>	0.05	$2.70 \times 10^4$

[a] The large error between this theoretical value and the experimental (*ie*. 0.7) is most probably due to the uncertainty in measuring the pH values at these extremely acidic solutions.

Table 3. Rate constants of <i>bis</i> -flavylium 1 and 2.				
	$k_{h} (s^{-1})$	$k_{-h} (M^{-1} s^{-1})$	$k_t / k_{-t} (s^{-1})$	<b>k</b> <sub>i</sub> / <b>k</b> <sub>-i</sub> (s <sup>-1</sup> )
1	6.05x10 <sup>-3</sup>	5.1x10 <sup>3</sup>	0.04 / 0.015	0.355 / 10 <sup>-5</sup>
2	1.5x10 <sup>-2</sup>	4.3x10 <sup>4</sup>	0.005 / 0.1	0.6 /2.2x10 <sup>-5</sup>

Finally, the data reported in Table 1 and Table 2 allow the construction of an energy-level diagram which is very useful to account for the details of the multistate, in particular to illustrate the photochemistry (Scheme 4). In the particular case of 1, the quantum yield at pH 3.9 is higher than at pH 5 because the forward reaction from  $C_c$  to  $AH^*/A$  is more efficient in the former. The thermal back reaction to restore the equilibrium is much slower in both cases. The species B and  $C_c$  are not easily accessible from the thermodynamic point of view. Moreover, at pH 5, the thermal back

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reactions are slower than at pH 3.9. This is due to the fact that at pH 5 there is more **A** in comparison with  $\mathbf{AH}^{\dagger}$ . As mentioned in the introduction, **A** is thus a kinetic product that retards the system to get the equilibrium. A similar situation is observed in the case of **2** (see Figure S3 in SI).



Scheme 4. Energy-level diagram of *bis*-flavylium 1. Ilustration of the pathways upon irradiation of the *trans*-chalcone (A) at pH 5, where the rate-determining step is the hydration. (B) The same at pH 3.9 where the hydration is faster.

# Conclusions

Symmetric bis-flavylium systems pose some interesting theoretical questions regarding the influence of the bridge on the chemical behavior of each terminal flavylium multistate. The present system proved to be stochastic in the sense that there is no measurable interaction between both flavylium multistates. The kinetics and thermodynamics of the bisflavylium is identical to a single flavylium multistate lacking of a cis-trans isomerization barrier. However, using the statistics, it is possible to calculate the distribution of the species at the molecular level. The  $pK_{a1}$  for removing one proton from the bis-flavylium is 0.3 units lower than the observed  $pK_a$ (macroscopic) while the  $pK_{a2}$  for removing the second proton is 0.3 units greater. The same is calculated for the equilibrium involving flavylium cation and trans-chalcone (macroscopic  $pK'_{a}$ ). Information of the kinetics at the molecular level can also be achieved mathematically. While the kinetic process after a direct pH jump is a single exponential ( $k_{obs}$ ), the rate at the molecular level to give one *trans*-chalcone is twice  $k_{obs}$  and the rate to give the second trans-chalcone from the remaining flavylium cation is the same as  $k_{obs}$ .

## **Experimental section**

## Synthesis

General - All starting materials were commercial and were used as received. Anhydrous solvents were freshly distilled before use or were obtained from the M. Braun Solvent Purification System (MB-SPS-800). The reactions were monitored by thin-layer chromatography carried out on silica plates (silica gel 60 F<sub>254</sub>, Merck) using UV-light for visualization. Column chromatographies were performed on silica gel 60 (0.040-0.063 mm, Merck) using mixtures of ethyl acetate and cyclohexane as eluents. - Evaporation of solvents were conducted under reduced pressure at temperatures less than 30°C unless otherwise noted. - Melting points (mp) were measured with a Stuart SMP30 apparatus in open capillary tubes and are uncorrected. - IR spectra were recorded with a Perkin-Elmer FTIR 1600 spectrometer (KBr disc) and values are reported in  $cm^{-1}$ . – <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on *i*) a Bruker Avance 300 spectrometer at 300 and 75 MHz, respectively, and on ii) a Bruker Avance 500 spectrometer at 500 and 125 MHz, respectively. Chemical shifts  $\delta$  and coupling constants J are given in ppm and Hz, respectively. Chemical shifts  $\delta$  are reported relative to residual solvent as an internal standard (acetonitrile-d<sub>3</sub>: 1.94 ppm for <sup>1</sup>H and 1.32/118.3 ppm for  ${}^{13}$ C; DMSO-d<sub>6</sub>: 2.50 ppm for  ${}^{1}$ H and 39.5 ppm for  ${}^{13}$ C).  ${}^{1}$ H multiplicities are designated by the following abbreviations: s = singlet, d = doublet, t = triplet, m = multiplet, quint. = quintuplet, b = broad. Carbon multiplicities were determined by DEPT135. - UV-Visible spectra were measured with a 8452A Hewlett-Packard spectrophotometer in the solvents indicated. Maximum absorption wavelengths  $\lambda^{max}$  and molar extinction coefficients  $\varepsilon$  are given in nm and mol<sup>-1</sup>dm<sup>3</sup>cm<sup>-1</sup>. – Electrospray (ESI) and Desorption Chemical Ionization (DCI) low/high-resolution mass spectra were obtained from the 'Service Commun de Spectroscopie de Masse' of the Plateforme Technique, Institut de Chimie de Toulouse.

Procedure for the synthesis of 4,4'-(propane-1,3diylbis(oxy))dibenzaldehyde 3: To a solution of 1,3dibromopropane (2.02 g, 10.0 mmol, 1.0 equiv.) in DMF (30 mL) were added 4-hydroxybenzaldehyde (2.93 g, 24.0 mmol, 2.4 equiv.) and K<sub>2</sub>CO<sub>3</sub> (4.15 g, 30.0 mmol, 3.0 equiv.). The mixture was heated at 100°C and stirred overnight (i.e., 12h). After cooling the reaction mixture to room temperature, H<sub>2</sub>O (30 mL) was added to the mixture in order to precipitate the reaction product. The mixture was filtered and the crude product was purified by column chromatography (cyclohexane/EtOAc - 9:1 to 7:3) to give 3 in pure form as a white solid (2.55 g, 90%).  $R_f = 0.4$  (cyclohexane/EtOAc, 7 : 3, v/v); mp: 131-132 °C; IR (KBr):  $v_{max}$  2 1690 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 9.87 (s, 2H), 7.88-7.85 (m, 4H), 7.17-7.14 (m, 4H), 4.27 (t, J = 6.6 Hz, 4H), 2.25 (quint., J = 6.3 Hz, 2H) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 191.3, 163.4, 131.8, 129.7, 114.9, 64.7, 28.3 ppm; MS (DCI<sup>+</sup>) m/z (%): 285 (25) [M-H<sup>+</sup>], 302 (100) [*M*-NH<sub>4</sub><sup>+</sup>].

Procedure for the synthesis of 1,1'-(4,4'-(propane-1,3diylbis(oxy))bis(4,1-phenylene))diprop-2-yn-1-ol 4: To a

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solution of **3** (1.02 g, 3.6 mmol, 1.0 equiv.) in THF (10 mL) was added a solution of commercial ethynylmagnesium bromide (0.5 M in THF, 19 mL, 9.4 mmol, 2.6 equiv.) at 0 °C. After addition of the Grignard reagent, the mixture was let warm and stirred at rt until the complete consumption of 3 (TLC). A saturated solution of  $\mathsf{NH}_4\mathsf{CI}$  was then added to the reaction mixture and the THF was evaporated under vacuum. The aqueous phase was extracted three times with EtOAc and the organic layers were washed with H<sub>2</sub>O and brine and then dried over Na<sub>2</sub>SO<sub>4</sub>. After evaporation of the solvent, the resulting crude product was purified by column chromatography (cyclohexane/EtOAc - 4:1) to give **4** as a pale yellow solid (1.08) g, 89%). R<sub>f</sub> = 0.2 (cyclohexane/EtOAc, 7 : 3, v/v); mp: 109-110 °C; IR (KBr):  $v_{max}$  2 3330, 3300, 2110 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 7.37-7.34 (m, 4H), 6.95-6.92 (m, 4H), 5.90 (d, J = 6.0 Hz, 2H), 5.27 (dd, J = 6.0, 2.4 Hz, 2H), 4.12 (t, J = 6.0 Hz, 4H), 2.16 (quint., J = 6.3 Hz, 2H) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 158.0, 134.1, 127.8, 114.1, 85.8, 75.6, 64.3, 62.0, 28.6 ppm; MS (DCl<sup>+</sup>) *m/z* (%): 319 (100) [*M*-OH<sup>+</sup>], 336 (15)  $[M^{+}]$ , 354 (30)  $[M-NH_{4}^{+}]$ .

Procedure for the synthesis of 1,1'-(4,4'-(propane-1,3diylbis(oxy))bis(4,1-phenylene))diprop-2-yn-1-one 5: To a solution of 4 (2.5 mmol, 1.0 equiv.) in EtOAc (15 mL) was added freshly prepared IBX<sup>18</sup> (2.8 g, 10 mmol, 4.0 equiv.) in one portion. The mixture was heated at 80 °C and stirred overnight (*i.e.*, 12h). After cooling the reaction mixture to room temperature, the mixture was filtered and EtOAc was evaporated under vacuum. The resulting crude product was purified by column chromatography (cyclohexane/EtOAc, 4:1 to 1:1) to give 5 as a yellow solid (815 mg, 98%).  $R_f = 0.3$  (cyclohexane/EtOAc, 7 : 3, v/v); mp: 145-146 °C; IR (KBr):  $V_{max}$  2 3230, 2090, 1635 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSOd<sub>6</sub>):  $\delta = 8.05-8.02$  (m, 4H), 7.16-7.13 (m, 4H), 5.00 (s, 2H), 4.28 (t, J =6.3 Hz, 4H), 2.25 (quint., J = 6.3 Hz, 2H) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta = 175.3$ , 163.7, 131.7, 129.0, 114.8, 84.5, 80.3, 64.8, 28.2 ppm; MS (DCl<sup>+</sup>) m/z (%): 333 (20) [*M*-H<sup>+</sup>], 350 (100) [*M*-NH<sub>4</sub><sup>+</sup>].

#### General procedure for the synthesis of bis-flavylium compounds

**1/2:** To a solution of resorcinol or phloroglucinol (1.0 mmol, 2.0 equiv.) and bis(arylethynylketone) **5** (0.5 mmol, 1.0 equiv.) in AcOH (2 mL) was added aqueous HPF<sub>6</sub> (0.5 mL, 60% in water). The solution, becoming immediately dark red, was stirred at rt for 48 hours. The resulting mixture was then plunged into Et<sub>2</sub>O (20 mL) where the *bis*-flavylium dihexafluorophosphate precipitated. The solid was recovered by filtration, washed with Et<sub>2</sub>O and finally dried under vacuum to give the expected dicationic species **1** and **2**, from resorcinol and phloroglucinol respectively.

#### bis(1'-((7-hydroxy)benzopyrilium)phenoxy)propane

173.4, 168.8, 167.4, 159.9, 155.0, 133.8, 133.4, 122.4, 122.3, 120.3, 117.5, 114.1, 103.9, 66.6, 29.4 ppm; UV/Vis (CH<sub>3</sub>CN/10% 1N HCl):  $\lambda^{max}(\epsilon) = 466$  (39000), 262 nm (16000 mol<sup>-1</sup>dm<sup>3</sup>cm<sup>-1</sup>); MS (ESI<sup>+</sup>) *m/z* (%): 259 (100) [*M*<sup>2+</sup>].

bis(1'-((5,7-dihydroxy)benzopyrilium)phenoxy) propane dihexafluorophosphate 2: Yellow solid (400 mg, 95%). IR (KBr):  $\nu_{max}$  ☑ 3410, 1640, 855 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, 1% TFA-d<sub>1</sub> in CD<sub>3</sub>CN):  $\delta$  = 9.04 (d, J = 8.8 Hz, 2H), 8.32-8.27 (m, 4H), 7.94 (d, J = 8.8 Hz, 2H), 7.21-7.18 (m, 4H), 6.96 (d, J = 2.2 Hz, 2H), 6.72 (d, J = 2.2 Hz, 2H), 4.38 (t, J = 6.2 Hz, 4H), 2.35 (quint., J = 6.2 Hz, 2H); <sup>13</sup>C NMR (125 MHz, 1% TFA-d<sub>1</sub> in CD<sub>3</sub>CN):  $\delta$  = 160.1, 159.6, 150.4, 133.2, 122.6, 117.7, 114.2, 111.5, 104.0, 96.9, 66.7, 29.9 ppm; UV/Vis (CH<sub>3</sub>CN/10% 1N HCl):  $\lambda^{max}$  ( $\varepsilon$ ) = 476 (21000), 416 (*sh*), 326 (6000), 278 nm (15000 mol<sup>-1</sup>dm<sup>3</sup>cm<sup>-1</sup>); MS (ESI<sup>+</sup>) *m/z* (%): 275 (100) [ $M^{2^+}$ ].

#### Characterization of full multistates sytems

**General** - Stock solutions of **1** and **2** were prepared in  $H_2O:EtOH 1:1$  (v:v) with HCl 0.1 M. The pH jumps were carried out by addition of base (direct) or acid (reverse) to equilibrated solutions of **1** or **2**. The pH of the solutions was adjusted by addition of HCl, NaOH, or Theorell and Stenhagen's universal buffer and pH was measured in a Radiometer Copenhagen PHM240 pH/ion meter. The final percentage of EtOH was always 50% (v:v).

**Measurements** - UV-Vis absorption spectra were recorded in a Varian Cary 100 Bio or Varian Cary 5000 spectrophotometers. The stopped-flow experiments were conducted in an Applied Photophysics SX20 stopped-flow spectrometer provided with a PDA.1/UV photodiode array detector.

**Data analysis and fitting** – All pKa values were determined from the absorbance versus pH experimental data. The observed rate constants were obtained fitting the absorbance versus time data to the integrated first order rate equation. The thermodynamic and kinetic results were globally fitted to the reported equations using the Solver tool in an Excel spreadsheet.

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**Two as one!** The network of chemical reactions involving two easy-to-prepare <u>bis\_flavyliving the Online</u> dications was explored from kinetic and thermodynamic viewpoints. By means of stopped flow and flash photolysis techniques, the so-investigated systems overall proved stochastic, thus revealing that no measurable interaction is taking place between the two multistate flavylium moieties.

