

# Ground- and excited-state properties of some naphthoflavyliums

Mourad Elhabiri, Paulo Figueiredo, Florian George, Jean-Paul Cornard, André Fougousse, Jean-Claude Merlin, and Raymond Brouillard

**Abstract:** A series of five, structurally related, substituted 2-phenyl-benzopyrylium (flavylium) salts were synthesized and characterized by NMR, absorption, and fluorescence techniques. Their hydration and deprotonation constants were obtained through thermodynamic and relaxation kinetic methods. Metallic complexation with the two compounds possessing a catechol group and its effect on the fluorescence intensity was also studied. The ground state properties of the five pigments are correlated with the theoretical data collected through AM1 molecular orbital calculations.

**Key words:** naphthoflavylum synthesis, hydration and deprotonation constants, fluorescence, metallic complexation, molecular orbital calculations.

**Résumé :** Cinq sels de 2-phényl-benzopyrylium (flavylium) ont été synthétisés et caractérisés par leurs spectres de RMN, d'absorption et de fluorescence. Les constantes d'hydratation et de déprotonation ont été déterminées par des méthodes thermodynamiques et cinétiques. La complexation de certains métaux avec les deux composés possédant un groupe catéchol et l'effet de ce type de complexe sur l'intensité de fluorescence ont également été étudiés. Les propriétés de l'état électronique fondamental des cinq pigments sont en bon accord avec les données théoriques obtenues par les calculs d'orbitales moléculaires selon la méthode AM1.

**Mots clés :** synthèse de cations naphthoflavylum, constantes d'hydratation et de déprotonation, fluorescence, complexation métallique, calcul d'orbitales moléculaires.

## Introduction

Diversely substituted 2-phenyl-benzopyrylium salts (henceforth referred to as flavylium salts) are frequently found in nature as the yellow to blue coloured pigments of most flowers and fruits (1, 2). They are the aglycone forms of a family of natural colourants, commonly known as anthocyanins (3), which have been consumed by humanity without any apparent unhealthy effects and have thus attracted the attention of numerous scientists for their use as an alternative to the hazardous synthetic dyes (4, 5). Unfortunately, the colourant properties of anthocyanins are somewhat unreliable under the physico-chemical conditions required for their possible use as food additives, namely mildly acidic pH values and interactions with an essentially aqueous environment (6–8). Indeed, in these conditions the flavylium form, which is usually stable at pH lower than 2, undergoes a hydration reaction (Scheme 1) leading to the formation of colourless forms. Moreover, both the coloured and colourless forms are susceptible to thermal

and photochemical degradation (9, 10). Several recent studies have, for these reasons, addressed the problem of the stabilization of such colourants in aqueous solution through the formation of molecular complexes with a wide array of natural compounds (1, 2, 11–23).

An alternative path for the obtention of stable coloured pigments is through laboratory synthesis of anthocyanin analogues possessing simple substituent groups, such as OH, CH<sub>3</sub>O, phenyl, etc., in key positions of the molecule (24–31) in order to provide not only the widest possible range of colours but also to protect the flavylium cation against nucleophilic attack in the pH domain referred to above (~3 to 5). Some of these synthetic flavylium compounds have revealed very interesting excited-state characteristics, namely fluorescence and photochromic properties (10, 25, 32–34).

With the objective of evaluating the influence on stability brought about by the introduction of different substituents in key positions of the flavylium skeleton, five compounds were synthesized: 2-benzo-naphtho[2,1-*b*]pyrylium chloride (1), 2-((3',4'-dihydroxy)-benzo)-naphtho[2,1-*b*]pyrylium chloride (2), 2-((3',4'-dihydroxy)-benzo)-3-*O*-methyl-naphtho[2,1-*b*]pyrylium chloride (3), 2-naphtho-naphtho[2,1-*b*]pyrylium chloride (4), and 2-benzo-naphtho[1,2-*b*]pyrylium chloride (5), Scheme 2. The extended conjugated  $\pi$ -systems of these molecules prompted us also to study their emissive properties and to correlate them with the degree of rigidity predicted by semi-empirical molecular orbital calculations for their molecular structures.

## Results

### Thermodynamic and kinetic measurements in aqueous media

The electronic absorption spectra of flavylium cations present,

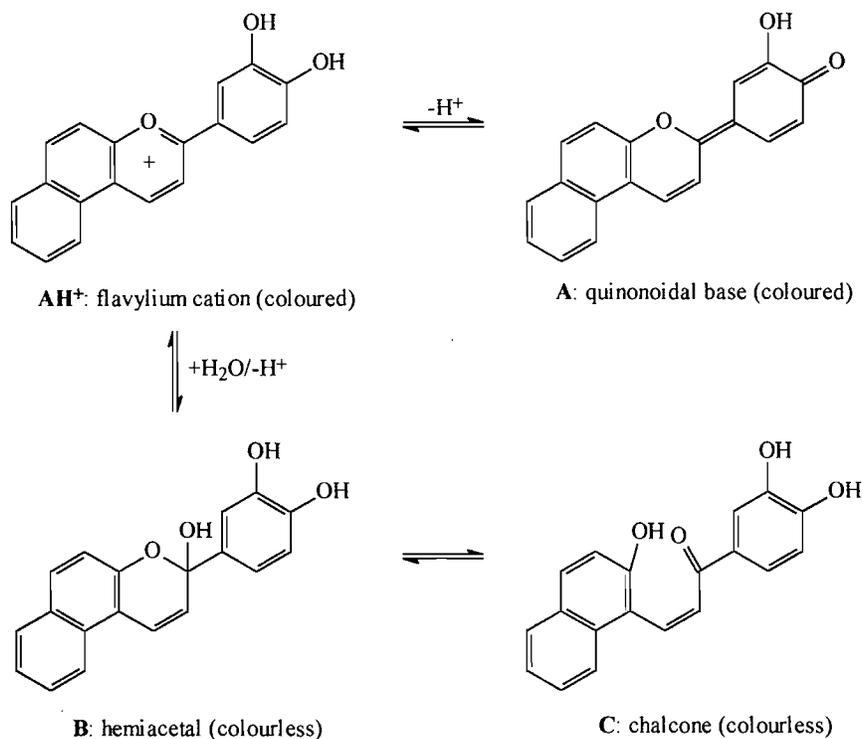
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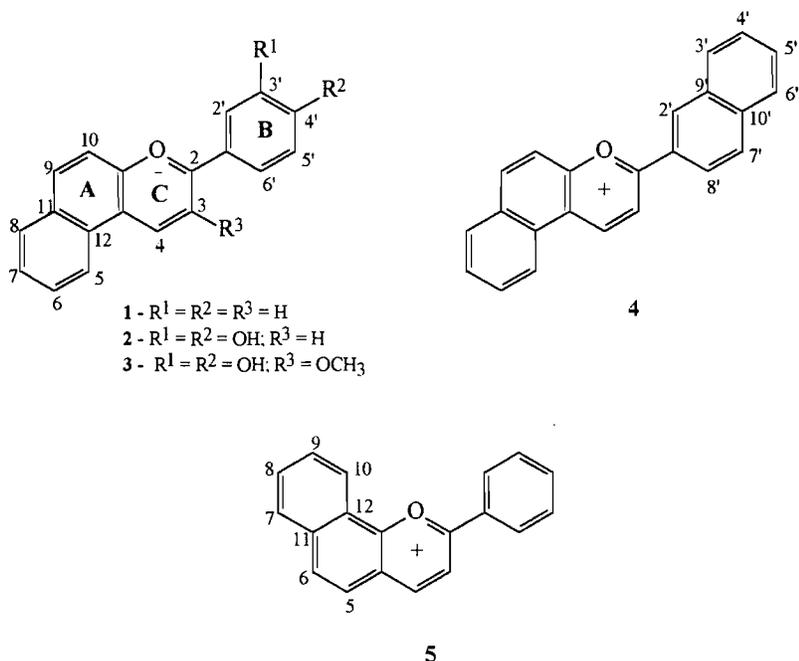
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Scheme 1.



Scheme 2.

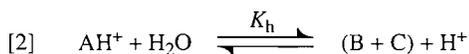
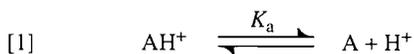
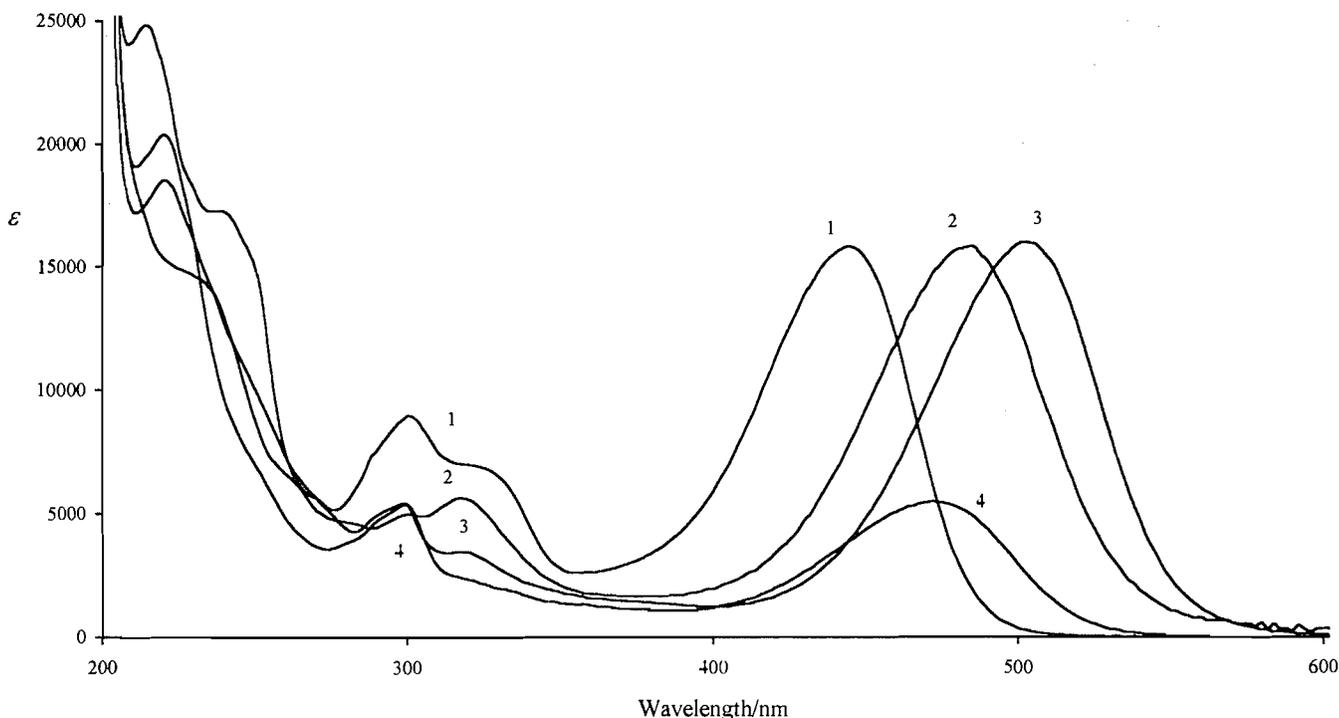


as a characteristic feature, a band in the visible spectral range, whose shape, maximum wavelength, and intensity are functions of the substitution pattern of the chromophoric system (26, 28, 31). Figure 1 reflects the spectral changes brought about by the introduction of different substituents in the same basic structure ( $\beta$ -naphthoflavylium, 1). Going from 1 to 3, it can be seen that the presence of the two hydroxyl groups in 3' and 4' produces a bathochromic shift from 444 nm to 484 nm

and that further addition of the 3-*O*-methyl produces an even larger shift to 502 nm. The shape and intensity of the absorbance bands are also affected by the presence of different types of substituents even when they are linked to the same positions in the basic flavylium molecule (cf. compounds 2 and 4).

The transformations undergone by these compounds in mildly acidic aqueous solution can be summarized by the equilibria

Fig. 1. Electronic absorption spectra of compounds 1–4. pH = 1.0. Aqueous solutions with 4% MeOH.



For the sake of simplicity, in eq. [2] the equilibria of hemiacetal (B) formation and subsequent ring opening to form the chalcone C are written as a whole since the second process occurs in a very fast step (35, 36). The increase in pH in aqueous solutions of these compounds with the subsequent attainment of the equilibrium described in eq. [2] produces significant modifications on the electronic spectra of the pigments (Fig. 2), notably a decrease in intensity of the visible band. This fact allows the obtention of a global value of  $K' = K_a + K_h$ , through a plot of  $D_0/(D_0 - D)$  as a function of  $[\text{H}^+]$ , according to eq. [3] (20, 21, 23). Such a plot will give a straight line with an intercept/slope ratio equal to  $K_h + K_a$ .

$$[3] \quad \frac{D_0}{D_0 - D} = \frac{K_h + K_a}{K_h + K_a(1 - r_A)} + \frac{[\text{H}^+]}{K_h + K_a(1 - r_A)}$$

In eq. [3]  $D_0$  represents the absorption of a strongly acidic (pH < 1) pigment solution,  $D$  the absorption of a solution at a given, less acidic, pH, and  $r_A$  stands for the ratio of the molar absorption coefficients  $\epsilon_A/\epsilon_{\text{AH}^+}$ .

An alternative and complementary method to obtain the values of the equilibrium constants is by performing pH jumps, from very acidic to less acidic pH values, in aqueous solutions of the pigments, and measuring the resulting exponential decay of the visible absorption, which essentially reflects the relaxation of the pH-dependent equilibrium [2] according to

an apparent first-order kinetics. The first-order rate constant  $k$  for [B] variation (defined by  $d \Delta [\text{B}]/dt = -k \Delta [\text{B}]$ ) is directly delivered by the spectrophotometer software and can be represented by

$$[4] \quad k = k_2[\text{H}^+] + \frac{k_1}{1 + K_a/[\text{H}^+]}$$

where  $k_1/k_2 = K_h$ , i.e.,  $k_1$  is the hydration rate constant and  $k_2$  the constant for the reverse process. Rearranging eq. [4] into eq. [5] allows the obtention of a  $(K_a + K_h + [\text{H}^+])/k$  vs.  $1/[\text{H}^+]$  straight-line plot with a slope equal to  $K_a/k_2$  and an intercept of  $1/k_2$ . This leads directly to the values for  $k_2$  and  $K_a$ , and through a combination with the results obtained from eq. [3],  $K_h$  and  $k_1$  immediately follow. The theoretical treatment that permits the obtention of equilibrium rate constants is given in full detail in ref. 21.

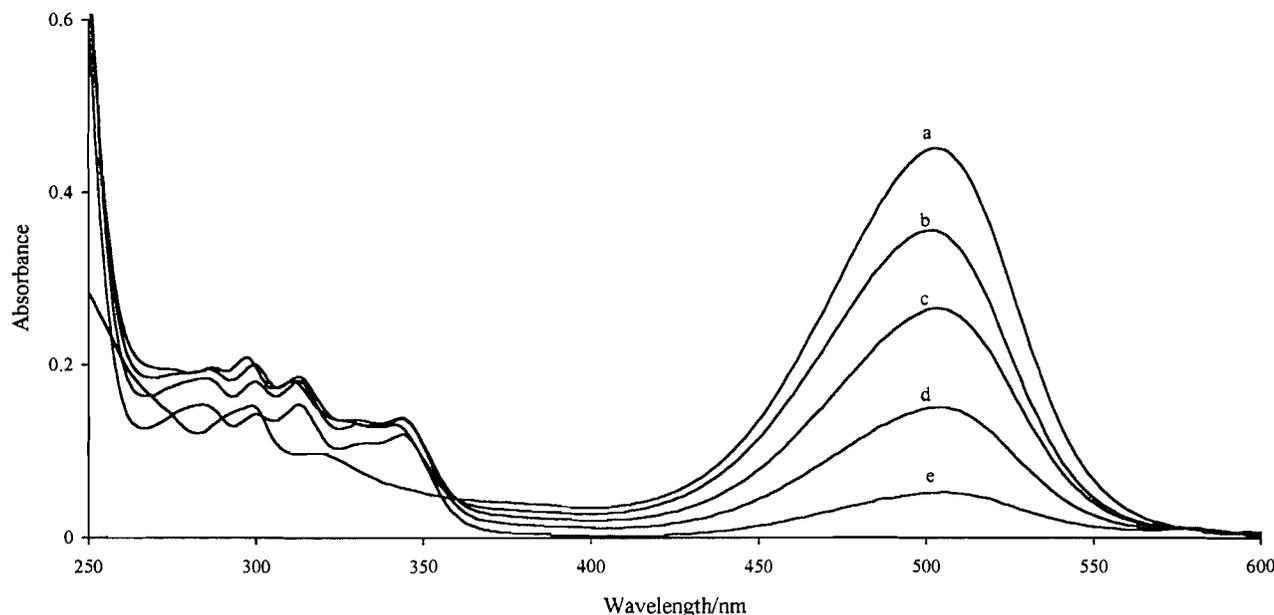
$$[5] \quad \frac{K_a + K_h + [\text{H}^+]}{k} = \frac{1}{k_2} + \frac{K_a}{k_2} \frac{1}{[\text{H}^+]}$$

Table 1 reports the calculated hydration and deprotonation constants for pigments 1–4.

#### Fluorescence properties

Most anthocyanins and some synthetic flavylum salts are known to exhibit fluorescence from all forms existing in mildly acidic aqueous solutions and even in some organic solvents (10, 25, 37, 38). However, all natural anthocyanins possess only very weak fluorescence intensities and poor fluorescence quantum yields, the same being true for the sim-

**Fig. 2.** Electronic absorption spectra of **3** as a function of pH. Concentration =  $2.8 \times 10^{-5}$  M;  $T = 25^\circ\text{C}$ . pH = 1.1 (a), 2.5 (b), 2.7 (c), 3.0 (d), and 3.4 (e).



**Table 1.** Thermodynamic and kinetic parameters for pigments **1–4** in aqueous solution.  $T = 25^\circ\text{C}$ .

Compound	$K_h$ ( $\text{p}K_h$ )	$K_d/\text{M}^{-1}$ ( $\text{p}K_d$ )
<b>1</b>	$1.02 \times 10^{-3}$ (2.99)	—
<b>2</b>	—	$1.41 \times 10^{-4}$ (3.85)
<b>3</b>	$1.51 \times 10^{-3}$ (2.82)	$6.76 \times 10^{-5}$ (4.17)
<b>4</b>	$4.32 \times 10^{-3}$ (2.365)	—

**Table 2.** Calculated parameters for compounds **1–5**. AM1 parametrization.

Compound	$S_2^N$	Torsion angle <sup>a</sup> ( $^\circ$ )
<b>1</b>	0.254	0.30
<b>2</b>	0.257	0.32
<b>3</b>	0.239	19.42
<b>4</b>	0.257	0.21
<b>5</b>	0.356	25.67

<sup>a</sup>Between rings C and B.

ple synthetic analogues studied so far. The extended conjugated systems of all pigments reported in this work and their planar molecular structures, especially in the cases of **1**, **2**, and **4** (Table 2), seemed to forecast a possible increase in emission intensity when compared to the above-mentioned pigments. Figure 3 compares the emission spectra of the flavylum forms of compounds **1–3** and **5**, where an influence of the substituents on emission intensity and wavelength is visible. Indeed, compounds **1**, **2**, and **3**, although absorbing at different wavelengths, emit at the same wavelength but the emission intensity of **2** is 500 times lower than that of **1**, and **3** is still less

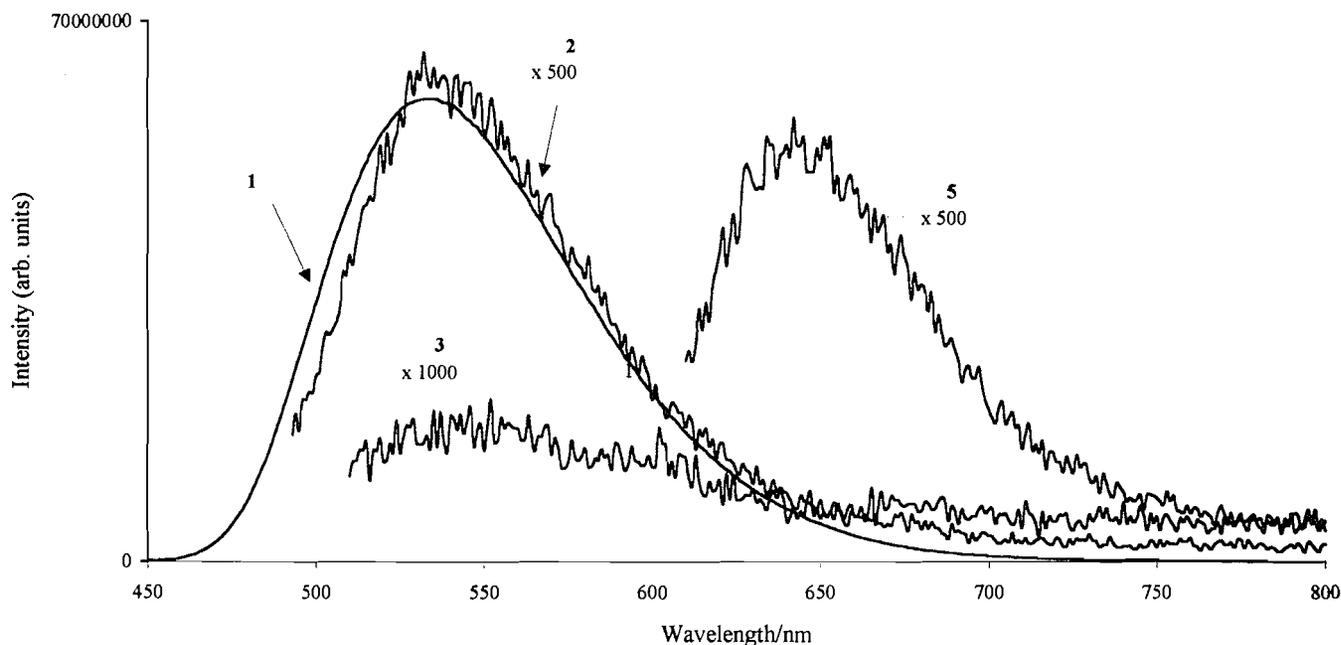
emissive. When compared to **1**, compound **5**, which can be considered as its isomer, is characterized by a strong Stokes shift in acidic methanolic solution, from 534 to 642 nm. Compound **4** also exhibits an emission band at 534 nm, its relative intensity being intermediate between those of **1** and **2**, in spite of being a more conjugated system.

#### Solvent effect

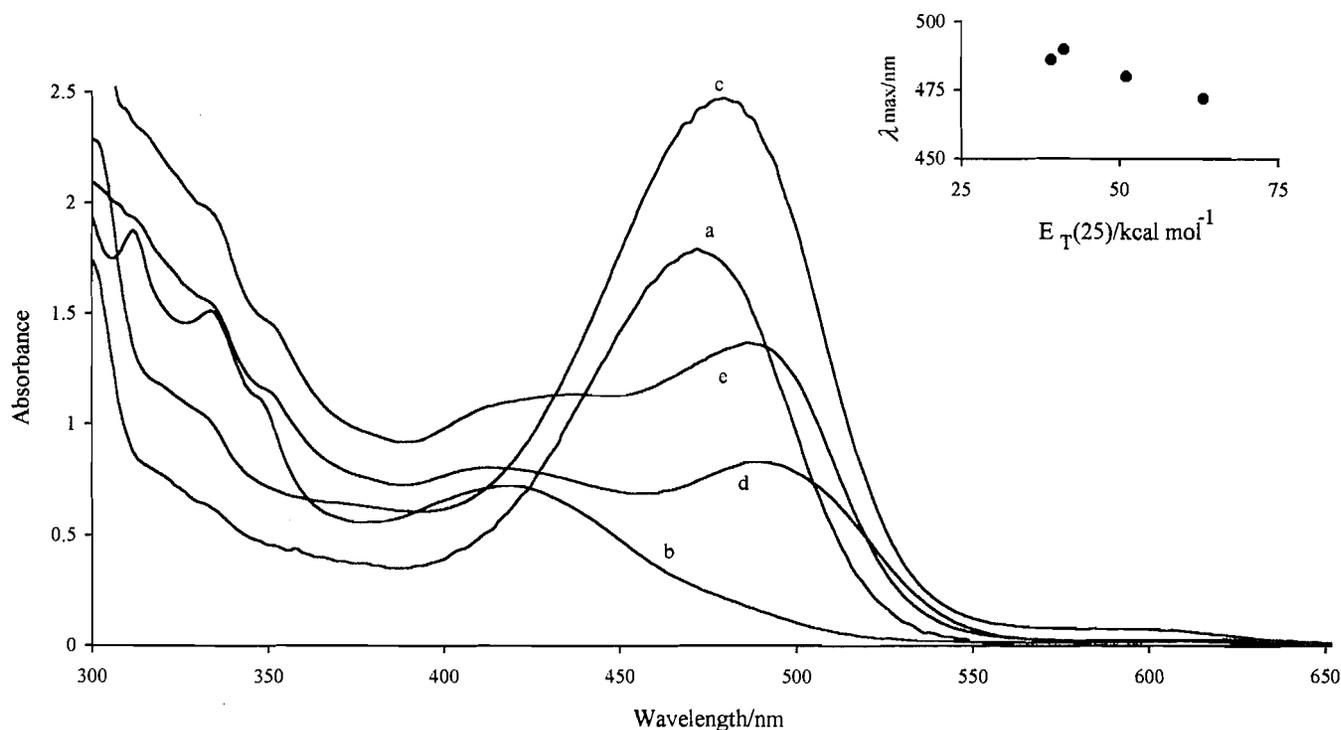
It is well known that flavylum forms of anthocyanic pigments present a negative solvatochromic behaviour, i.e., a displacement of the visible absorption band towards longer wavelengths with a reduction in medium polarity (39). Absorption and fluorescence emission spectra of pigments **1**, **4**, and **5** were thus performed in several solvents, ranging in polarity from chloroform to water, to assess the solvent influence on absorption and emission wavelengths and intensities of this particular type of flavylum salts. The solvents used were, in order of increasing polarity, chloroform, dichloromethane, formic acid, methanol, and water/HCl. The changes in absorption spectra caused by variation in medium polarity in **4** are presented in Fig. 4. Considerable changes in band shape can be observed, first the disappearance of the typical flavylum absorption and the clear vibrational structure at lower wavelength in methanol, which is also slightly perceptible both in chloroform and dichloromethane. In the cases of chloroform and dichloromethane, the typical bathochromic shift due to medium polarity on the flavylum absorption band is accompanied by the formation of a shoulder at ca. 420 nm. The two other pigments (**1** and **5**) show similar behaviour, notably a bathochromic shift with decreasing medium polarity and the appearance of structured absorbance bands in methanolic solution.

In what concerns fluorescence emission, a hypsochromic shift is observed when decreasing the medium polarity. Furthermore, strong differences of behaviour can be observed between the two isomers **1** and **5**, when comparing their fluo-

**Fig. 3.** Fluorescence emission spectra of **1**,  $\lambda_{EX} = 445$  nm; **2**,  $\lambda_{EX} = 485$  nm; **3**,  $\lambda_{EX} = 500$  nm; and **5**,  $\lambda_{EX} = 600$  nm. Acidic methanolic solutions.



**Fig. 4.** Absorption spectra of **4** in  $H_2O$ , pH = 1.0 (a),  $CH_3OH$  (b),  $HCOOH$  (c),  $CH_2Cl_2$  (d), and  $CHCl_3$  (e).  $3.25 \times 10^{-4}$  M,  $T = 25^\circ C$ . Inset: variation of  $\lambda_{max}$  as a function of medium polarity.



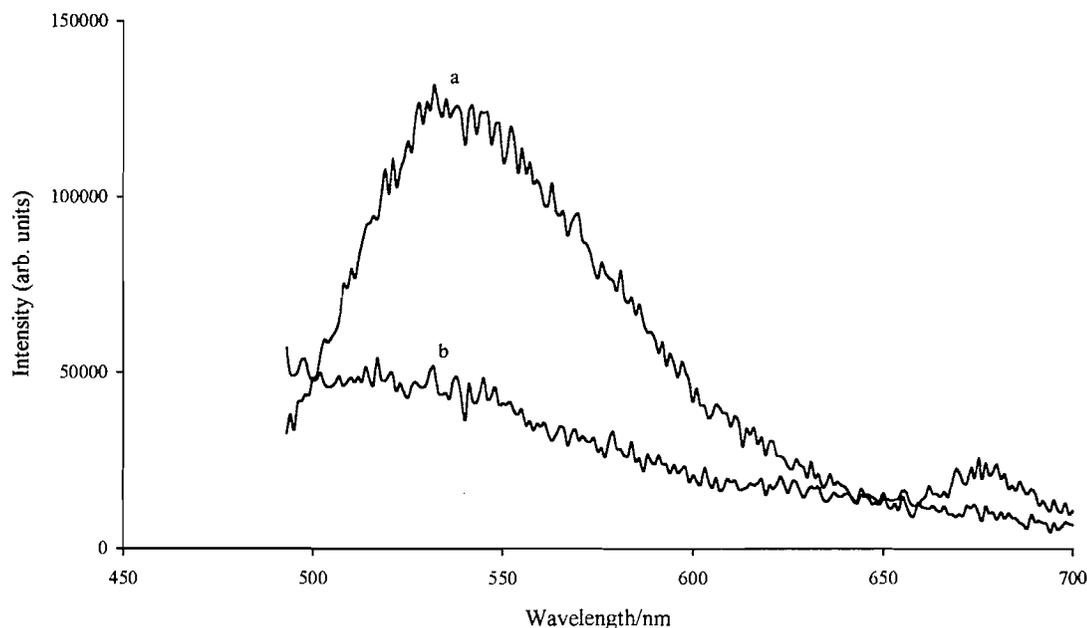
rescence properties in solvents of different polarity. In methanol both **1** and **5** show low-intensity emission bands, when excited at the maximum visible wavelength of their respective flavylum forms, this intensity strongly increasing when a few drops of concentrated formic acid are added to the solution. But in dichloromethane solutions, while **1** produces emission

bands more intense than those found in acidic methanol, **5** displays only very weak fluorescence bands.

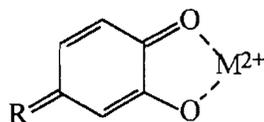
#### Photochromic behaviour

Certain synthetic flavylum salts, in mildly acidic aqueous media ( $3 < pH < 4$ ), have shown interesting photochromic

Fig. 5. Fluorescence emission spectra of **2** in H<sub>2</sub>O, pH ≈ 4.0, (a) without Al<sup>3+</sup>, and (b) with Al<sup>3+</sup>.



Scheme 3.



properties with either gain or loss of colour under light irradiation and the inverse behaviour in the dark (10, 32, 34). This prompted us to investigate the photochromic behaviour of the five naphthoflavyliums here reported. Yet, only pigment **3** revealed a manifest photochromic effect with loss of colour when submitted to irradiation, and a partial colour recovery in the dark. Colour recovery was not complete since side reactions of thermal and photo degradation occur.

#### Metallic complexation

Chelation between some small di- and trivalent metals and anthocyanins possessing a catechol group in ring B has been reported (1, 22, 40, 41) and discussed on the basis of phenolic proton(s) loss to form a quinonoidal structure (Scheme 3), which will be responsible for the bathochromic shift always observed. To our knowledge, no work has been done on the fluorescence properties of these metal complexes. This prompted us to investigate the fluorescence emission changes when europium(III) or aluminium(III) salts are added to stabilized solutions of compounds **2** and **3**.

For both compounds, the addition of a solution of AlCl<sub>3</sub> in large excess produces a bathochromic shift of 60 nm in the visible absorption band and a strong decrease of the fluorescence intensity at 534 nm characteristic of the flavylum cation, which is in accordance with the deprotonation model proposed above. Moreover, a faint emission band appears at 675 nm (Fig. 5) and 690 nm, respectively, for **2** and **3**, which becomes more intense when the solution is excited at the new maximum wavelength of the complex.

## Discussion

### Thermodynamic and kinetic measurements in aqueous media

The spectral changes shown in Fig. 1, namely, the bathochromic shifts observed between pigments **1**, **2**, and **3**, can be attributed to the increase in electron donor strength of the substituting OH and OCH<sub>3</sub> groups. A similar phenomenon is known to occur with natural, largely substituted, anthocyanins, which tend to absorb at longer wavelengths than the parent aglycones (2).

The equilibrium constants obtained through application of eqs. [3] and [5] are presented in Table 1. No values are displayed for pigment **5**, due to its insolubility in aqueous solution. In the cases of molecules **1** and **4**, which possess no OH group and thus cannot permit deprotonation (eq. [1]),  $K_h$  is directly obtained from eq. [3] and is equal to  $K'$ .

From the analysis of Table 1, it can be inferred that compound **4** is the most susceptible to hydration (lower  $pK_h$ ), while **2** seems to suffer only a negligible water attack. This is evidenced by the fact that we can obtain the value for only a single equilibrium constant and is further stressed by the subtraction of the flavylum contribution (37) from the absorption spectra as a function of the pH. This results only on the rise of a new band with a maximum wavelength at ca. 555 nm, which is the same maximum wavelength obtained for a fresh solution of **2** at pH 7, where the quinonoidal base is the dominant species. Moreover, an isosbestic point at ca. 530 nm is observed, supporting the existence of only two forms in equilibrium. Since no other absorptions, attributable to the hemiacetal and (or) the chalcone forms, appear as a result of the subtraction, the value found for the equilibrium constant is assigned to  $K_a$ .

The greater or lower reactivity towards nucleophilic water attack can be correlated with the charge density in C-2 (given

by the parameter  $S_2^N$ , obtained through molecular orbital calculations. Examination of Table 2 allows the conclusion that an increase in  $S_2^N$  is accompanied by a diminution in  $pK_h$ . This confirms that, for these naphtho derivatives, the hydration reaction occurs in the same manner as that reported for common flavylum salts (2, 28). There is, however, an exception represented by compound **4**, which presents only a small increase in  $S_2^N$  relative to **1**, but has a considerably lower  $pK_h$ . A tentative explanation for this behaviour is given by taking into account the large absolute hardness value ( $\eta$ ) that was computed, through molecular orbital calculations, for pigment **1** (3.31 eV) as compared to the value obtained for compound **4** (3.05 eV). This parameter is defined as  $\eta = (E_{LUMO} - E_{HOMO})/2$  and is usually a reliable criterion for predicting reactivity, i.e., the larger the LUMO–HOMO gap the lower the reactivity of the molecule (31). The greater reactivity of **4** may therefore reflect a less aromatic character of this compound relative to **1** in spite of the presence of an additional benzene fragment in **4** (see below the discussion of the fluorescence properties).

### Fluorescence properties

The spectra depicted in Fig. 3 demonstrate the influence that is brought about by different substituents on emission intensity and wavelength. The difference in emission intensity among pigments **1–3** may be due to the formation of a twisted intramolecular charge transfer state (TICT) in the excited state, caused by the presence of the OH groups in the case of **2** and further enhanced by the loss of coplanarity in **3** (Table 2). Some authors have already verified that, for similar molecules, the rigidity and consequent coplanarity of the moiety connected to C-2 of the benzopyrylium fragment has a marked influence on their fluorescence properties (25, 42–44). The formation of this TICT state can be viewed as a deactivation channel for the fluorescence emission.

The observed Stokes shift between **1** and **5** (Fig. 3) is attributable to the strong electron-donating properties of the benzyl moiety on position 11 of the latter (25). A strong bathochromic shift from 444 nm to 600 nm is also observed on the absorption spectra of these two compounds.

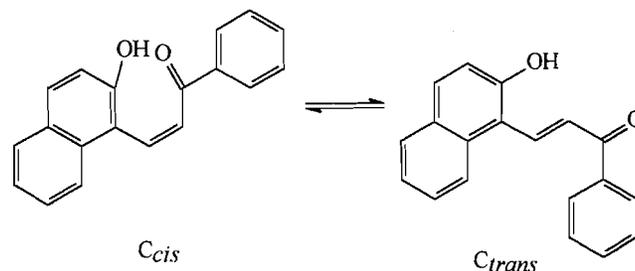
The structure of compound **4** (Scheme 2) leads us to anticipate a strong fluorescence emission. However, this is not observed. As stated before, it exhibits an emission intensity lower than **1**. This molecule is a good example that it is not simply by combining a great number of benzene fragments that a molecule becomes more aromatic, as demonstrated by the smaller  $E_{LUMO} - E_{HOMO}$  gap (28) obtained from molecular orbital calculations applied to **4**. This gap, which is a good measure of the aromaticity of a molecule, was computed as 3.05 eV, compared to 3.31 eV for **1**.

### Solvent effect

The insert of Fig. 4 depicts the existing correlation between  $E_T$  at 25°C (45) and the maximum wavelength of compound **4** in several solvents, the same relation holding for the two other compounds tested (**1** and **5**). This relation reflects the negative solvatochromic behaviour of these three naphthoflavyliums.

The existence of vibrational bands in the UV region of the absorption spectra, observed for compounds **1**, **4** (Fig. 4), and **5** in methanolic solution, should have their origin in the formation of a methoxy adduct to position 2 (see Scheme 2) of the chromophore, since when such a solution is quickly acidified

Scheme 4.



to pH 1 a spectrum similar to the one observed in aqueous acidic solution is obtained. This methoxy adduct will thus be a methanolic analogous to the hemiacetal formed upon hydration of the flavylum cation, in aqueous medium.

The appearance of a shoulder next to the typical visible absorption band, in chloroform and dichloromethane solutions (Fig. 4), may be caused by a rigidization of the molecular structure in these less polar solvents.

The hypsochromic shift observed in fluorescence emission spectroscopy when the medium polarity is lowered, which appears to be in contradiction with the bathochromic shift observed in absorption spectra, can be explained by a greater delocalization of the positive charge in the excited state than in the ground state. This leads to a smaller stabilization of  $S_1$  relative to  $S_0$  by solvents of high polarity. Since the polarizability of  $S_1$  is greater than that of  $S_0$ , less polar solvents stabilize the latter and result in a hypsochromic shift of the emission wavelength.

Finally, it may be the presence of a substituent in position 11, in pigment **5**, responsible for the creation of a deactivation channel, more effective in less polar solvents, that would account for the low intensity of emission displayed by this compound in dichloromethane solution, when compared to its isomer **1**.

### Photochromic behaviour

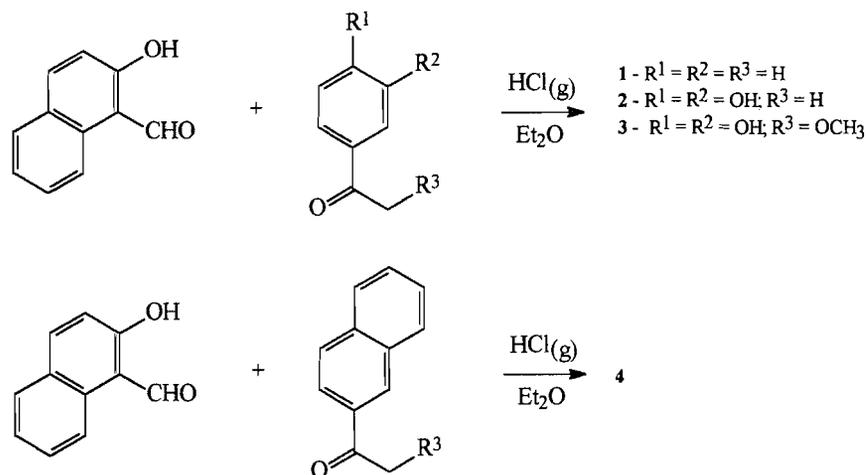
The photochromic properties here reported for compound **3**, in mildly acidic aqueous solutions, seem to be typical of some 3-substituted flavylum based pigments that form the colourless *cis*-chalcone (cf. Scheme 4) as the more stable isomer in the ground state (32, 46). Light irradiation allows us to overcome the energy barrier necessary to displace the equilibrium towards the *trans* isomer (also colourless). The *trans*-chalcone, thus formed in the excited state as the more stable isomer, does not possess the ability to cyclize and reform the flavylum cation according to the set of equilibria depicted in Scheme 1. The displacement of these equilibria towards *trans*-chalcone accumulation results therefore in a loss of colour. This reaction is thermally reversed in the dark.

The inverse process does also occur with some, 3-unsubstituted, flavylum compounds (34), i.e., stabilization of the *trans*-chalcone in the ground state with the irradiation producing a *trans*→*cis* photoisomerization with consequent recyclization of the *cis*-chalcone to reform the flavylum cation. This reaction is also reversed in the dark.

### Metallic complexation

The new emission bands observed at 675 and 690 nm (Fig. 5) upon excitation of aqueous solutions of, respectively, compounds **2** and **3** in the presence of Al(III), although having the

Scheme 5.



same maximum wavelength as those of a fresh solution of the pigments at neutral pH (where the quinonoidal base is the predominant form in solution), are less intense. This fact may be explained by a quenching process as a consequence of aluminium complexation. Such quenching effect is increased when europium(III) replaces aluminium, since in this case no new emission band accompanies the decrease in the flavylum cation emission.

## Conclusion

Ground-state properties of a series of structurally related pigments are shown to be strongly dependent on the substitution pattern and on the greater or lesser coplanarity and aromaticity of the molecule. Commonly, the stronger the electron donor character of the substituent(s), the greater the observed bathochromic shift. The twisting of the B ring relative to the rest of the molecule seems to act in the same direction. Concerning excited-state properties, it is the substitution pattern that seems to play the most significant role. Notably, substitution in position 11 seems to have a profound impact, namely, producing a significant Stokes shift. The other substitution patterns studied do not affect the emission wavelength, but the fluorescence intensity strongly decreases with the presence of donor substituents, through the possible formation of a nonfluorescent TICT state, indicating that nonsubstituted and planar compounds, like **1**, are good candidates for highly aromatic and fluorescent molecules.

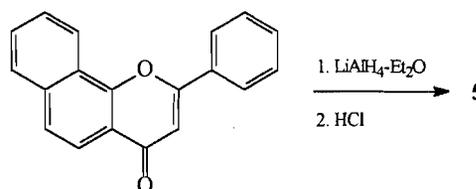
## Computational

Molecular orbital calculations, using AM1 parametrization (47) were performed on a Escom P100 PC, using the HyperChem program (version 4, Hypercube, Inc., Ont. Canada).

## Experimental

Fast atom bombardment (FAB) mass spectrometry was carried out either on a LBK 9000S or on a Thomson THN 208 instrument. Electron-spray ionization (ESI) mass spectrometry was carried out with a Bio-Q quadrupole instrument. NMR spectra were recorded at 27°C on a Bruker AM-400 spectrometer operating at 400.14 MHz for  $^1\text{H}$  and on a Bruker WP-200SY operating at 50 MHz for  $^{13}\text{C}$ . Standard pulse frequencies and

Scheme 6.



phase cycling scheme (48) were used to record  $^1\text{H}$ - $^1\text{H}$  COSY (49, 50). Samples (4–8 mg/mL) were prepared in  $(\text{CD}_3)_2\text{SO}-\text{CF}_3\text{COOD}$  (98:2),  $\text{CD}_3\text{CN}-\text{CF}_3\text{COOD}$  (98:2). Residual signals of the solvent were used as internal chemical shift reference. Electronic absorption spectra were acquired using a Hewlett-Packard diode-array spectrometer fitted with a quartz cell ( $d = 1$  cm) equipped with a stirring magnet. A constant temperature in the cell was obtained by use of a Lauda water-thermostated bath. The temperature was measured in the spectrometer cell with a Cormak thermocouple and was kept at  $25 \pm 0.1^\circ\text{C}$  throughout this work. Fluorescence emission spectra were recorded on a SPEX F111 Fluorolog and on a molecular microprobe operating in a way that favours observation of the flavylum emission bands (51). Solutions used in fluorescence measurements always had an absorbance of 0.2 at the excitation wavelength. The pH was measured with a Metrohm 654 pH-meter equipped with a micro-electrode. Milli-Q water was used in all aqueous sample preparations. All other solvents were of analytical grade except for fluorescence measurements where spectroscopic grade solvents were used. 2'-O-Methyl-3,4-dihydroxyacetophenone and 3,4-dihydroxyacetophenone were prepared from 2'-chloro-3,4-dihydroxyacetophenone, according to ref. 22. All other reagents used throughout the synthesis were of synthetic or analytical grade and used as received.

This particular class of benzopyrylium salts was prepared by two different methods. The first consists of acidic condensation of an appropriate aldehyde with a ketone (52) (Scheme 5). In the second, the synthesis proceeds through the reduction of the carbonyl group of a flavone by  $\text{LiAlH}_4$  followed by a treatment with hydrochloric acid (Scheme 6).

## General procedure for compounds 1–4

An equimolar solution of the suitable ketone and aldehyde

precursors was vigorously stirred in distilled diethyl ether oxide. Gaseous hydrogen chloride was gently bubbled (3 h) into the mixture to favour solubilization when the initial products presented weak solubilities. Precipitation of coloured crystals occurred after some minutes and was completed by keeping the reaction mixture at  $-20^{\circ}\text{C}$  overnight. Crystals were washed several times with distilled diethyl ether oxide and dried under vacuum.

#### 2-Benzonaphtho[2,1-b]pyrylium chloride (1)

Total yield is 55%. UV-VIS ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (nm): 244, 301, 330, 456.  $^1\text{H NMR}$  ( $\text{CD}_3\text{CN/TFA}$ )  $\delta$  (ppm): 10.74 (1H, d,  $J = 9.2$  Hz, H4), 9.51 (1H, d,  $J = 9.2$  Hz, H3), 9.12 (2H, d,  $J = 2.2/7.5$  Hz, H2', H6'), 8.63–8.32 (5H, complex signals, H3', H4', H5', H6, H7), 9.49 (1H, d,  $J = 9.0$  Hz, H5), 8.84 (1H, d,  $J = 9.3$  Hz, H8), 8.82 (1H, d,  $J = 9.2$  Hz, H9), 9.37 (1H, d,  $J = 9.3$  Hz, H10). ESI-MS  $m/z$ : 257.1 for  $\text{C}_{19}\text{H}_{13}\text{O}$ .

#### 2-((3',4'-Dihydroxy)benzo)naphtho[2,1-b]pyrylium chloride (2)

Total yield is 55%. UV-VIS ( $\text{HCl}$  0.1 M)  $\lambda_{\text{max}}$  (nm): 240, 300, 320, 486.  $^1\text{H NMR}$  ( $\text{DMSO/TFA}$  signals assigned by  $^1\text{H}-^1\text{H}$  COSY)  $\delta$  (ppm): 10.07 (1H, d,  $J = 9.2$  Hz, H4), 8.98 (1H, d,  $J = 9.2$  Hz, H3), 8.17 (1H, dd,  $J = 2.3/8.5$  Hz, H6'), 8.02 (1H, d,  $J = 2.2$  Hz, H2'), 7.13 (1H, d,  $J = 8.6$  Hz, H5'), 8.85 (1H, d,  $J = 9.3$  Hz, H5), 7.97 (1H, t,  $J = 8.0$  Hz, H6), 7.87 (1H, t,  $J = 8.2$  Hz, H7), 8.24 (1H, d,  $J = 9.4$  Hz, H8), 8.26 (1H, d,  $J = 9.3$  Hz, H9), 8.71 (1H, d,  $J = 9.3$  Hz, H10). FAB-MS  $m/z$ : 289.3 for  $\text{C}_{19}\text{H}_{13}\text{O}_3$  ( $100\text{ M}^+$ ), 579.2 (observation of a dimer, which can also be noticed by a deviation to the law of Beer-Lambert, when concentrations superior to  $2 \times 10^{-5}$  M were used).

#### 2-((3',4'-Dihydroxy)benzo)-3-O-methylnaphtho[2,1-b]pyrylium chloride (3)

Total yield is 75%. UV-VIS ( $\text{HCl}$  0.1 M)  $\lambda_{\text{max}}$  (nm): 240, 300, 320, 504.  $^1\text{H NMR}$  ( $\text{DMSO/TFA}$  signals assigned by  $^1\text{H}-^1\text{H}$  COSY)  $\delta$  (ppm): 9.45 (1H, s, H4), 8.27 (1H, dd,  $J = 2.3/8.8$  Hz, H6'), 8.10 (1H, d,  $J = 2.3$  Hz, H2'), 7.07 (1H, d,  $J = 8.7$  Hz, H5'), 4.34 (3H, s), 7.04 (1H, d,  $J = 7.9$  Hz, H5), 7.81 (1H, t,  $J = 8.1$  Hz, H6), 7.90 (1H, t,  $J = 8.1$  Hz, H7), 8.16 (1H, d,  $J = 9.0$  Hz, H8), 8.14 (1H, d,  $J = 9.0$  Hz, H9), 8.43 (1H, d,  $J = 9.0$  Hz, H10). ESI-MS  $m/z$ : 319.34 for  $\text{C}_{20}\text{H}_{15}\text{O}_4$ .

#### 2-Naphthonaphtho[2,1-b]pyrylium chloride (4)

Total yield is 60%. UV-VIS ( $\text{CH}_3\text{COOH}$ )  $\lambda_{\text{max}}$  (nm): 252, 300, 476.  $^1\text{H NMR}$  ( $\text{CD}_3\text{CN/TFA}$ )  $\delta$  (ppm): 10.74 (1H, d,  $J = 9.2$  Hz, H4), 9.51 (1H, d,  $J = 9.2$  Hz, H3), 9.12 (2H, d,  $J = 2.2/7.5$  Hz, H2', H6'), 8.63–8.32 (5H, complex signals, H3', H4', H5', H6, H7), 9.49 (1H, d,  $J = 9.0$  Hz, H5), 8.84 (1H, d,  $J = 9.3$  Hz, H8), 8.82 (1H, d,  $J = 9.2$  Hz, H9), 9.37 (1H, d,  $J = 9.3$  Hz, H10). FAB-MS  $m/z$ : 307.1 for  $\text{C}_{23}\text{H}_{15}\text{O}$  ( $100\text{ M}^+$ ), 615.2 (once again observation of a dimer).

#### 2-Benzonaphtho[1,2-b]pyrylium chloride (5)

$\alpha$ -Naphthoflavone (200 mg, 0.73 mmol) was dissolved in 10 mL of a mixture of diethyl ether oxide and toluene and 1.1 equivalent of  $\text{LiAlH}_4$  (20 mg) was added. The solution was kept under reflux for 2 h, diluted with ethyl acetate, and washed with two portions (each 50 mL) of water. The organic phase was then acidified with hydrochloric acid. The solution turned green and was concentrated under vacuum. The pyrylium salt was purified on a silica column, first with  $\text{CHCl}_3$  as

eluent and after with  $\text{CHCl}_3$ - $\text{CH}_3\text{COOH}$  (95.5). Total yield is 35%. UV-VIS ( $\text{CH}_3\text{COOH}-\text{CHCl}_3$ , 1:1)  $\lambda_{\text{max}}$  (nm): 244, 297, 383, 425, 604.  $^1\text{H NMR}$  ( $\text{CD}_3\text{CN/TFA}$ )  $\delta$  (ppm): 10.54 (1H, d,  $J = 9.2$  Hz, H4), 8.84 (1H, d,  $J = 9.2$  Hz, H3), 8.32–7.71 (10H, complex signals, H2', H3', H4', H5', H6', H6, H7, H8, H9, H10), 8.79 (1H, d,  $J = 9.0$  Hz, H5). FAB-MS  $m/z$ : 257.1 ( $100\text{ M}^+$ ) for  $\text{C}_{19}\text{H}_{13}\text{O}$ .

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