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Unidirectional Switching between Two Flavylium Reaction Networks by the Action of Alternate Stimuli of Acid and Base.

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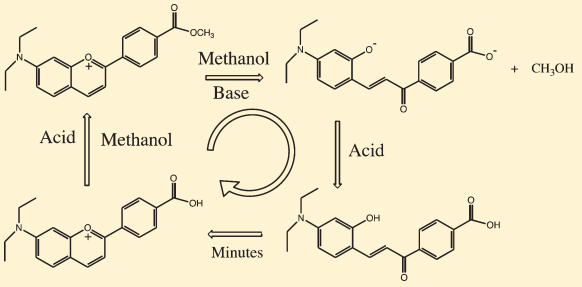
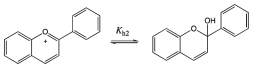
ABSTRACT: The introduction of an ester group in the flavylium core allowed the reversible conversion between two different flavylium compounds each one exhibiting its own reaction network. An unidirectional switching cycle between 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium and 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium was achieved by means of alternate acid and base stimuli. Addition of base to a methanolic solution of the ester derivative gives rise to the *trans*-chalcone of the parent carboxylic acid, which upon acidification of the solution forms the respective flavylium cation. This species esterifies under very acidic conditions to restore the original methyl ester derivative. The chemical reaction networks of both compounds were fully characterized from their thermodynamic and kinetic aspects, by a series of pH jumps followed by UV−vis absorption and emission spectroscopy, stopped flow and ^{1}H NMR. The crystal structure of the *trans*-chalcone of the ester derivative was unveiled showing a supramolecular structure involving hydrogen bonding.



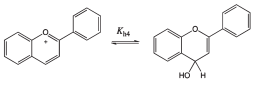
INTRODUCTION

The pH-dependent network of flavylium compounds is well established in particular for those lacking hydroxyl substituents where formation of B4 has been observed. In the case of the flavylium cation (lacking of substituents),[1−3] in moderately acidic to neutral aqueous solutions, a sequence of reactions takes place, starting from the hydration in position 2 to give B2, eq 1,



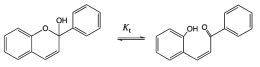
$AH^+ + 2H_2O \rightleftharpoons B2 + H_3O^+$ K_{h2}=9.8x10^{-4} M (1)

or in position 4 to form B4, eq.(2)



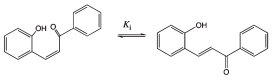
$AH^+ + 2H_2O \rightleftharpoons B4 + H_3O^+$ K_{h4}=1.2 x10^{-5} M (2)

The hemiketal B2 leads the *cis*-chalcone, eq.(3)



$B2 \rightleftharpoons Cc$ K_t=0.06 (3)

and finally the *cis*-chalcone isomerizes to the *trans*-isomer, eq.(4)



$Cc \rightleftharpoons Ct$ K_i=400 (4)

With $K_n = \frac{k_n}{k_{-n}}$ and n=h2, h4, t and i.

It is easy to show[4] that eq 1−4 behave as a single acid base equilibrium, eq 5

$$AH^+ + H_2O \rightleftharpoons CB + H_3O^+$$
$$K'_a = K_{h2} + K_{h4} + K_{h2}K_t + K_{h2}K_tK_i \quad (5)$$

being [CB] = [B2] + [B4] + [Cc] + [Ct].

When the *cis*−*trans* isomerization is much slower than the other reactions a pseudo stationary state can be defined by means of eq 6, and behaving also as a single acid−base equilibrium

$$AH^+ + H_2O \rightleftharpoons CB\hat{} + H_3O^+$$
$$K_a = K_{h2} + K_{h4} + K_{h2}K_t \quad (6)$$

being [CB^] = [B2] + [B4] + [Cc].

One useful way to the comprehension of the pH dependent thermodynamic and kinetics of the flavylium network of chemical reactions is the construction of an energy level diagram as the one shown in Scheme 1.[5]

At acidic medium the thermodynamic stable species is the flavylium cation. As the pH raises B2, B4 and Cc are formed as transient species, but the final equilibrium involves essentially the *trans*-chalcone.

The kinetics of the (not substituted) flavylium system were also reported, Scheme 2.[1−3]

After the work of McClelland and Gedge,[2] it was established that the terms in gray are due to the catalysis, and thus after a pH

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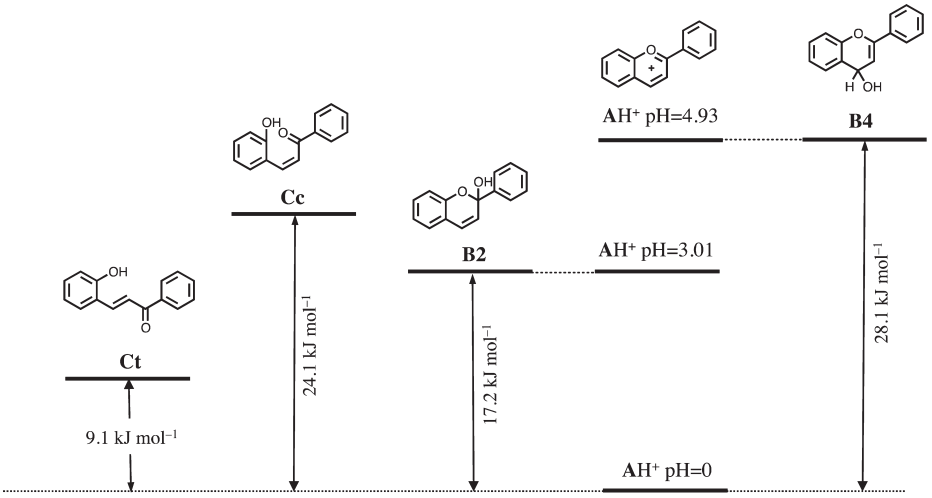
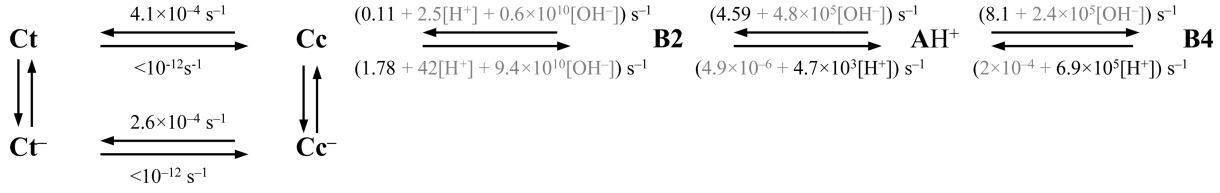
Scheme 1



Scheme 2



jump from 1 (flavylium cation) to for example pH = 5, B2 and B4 are formed with rates approximately 5 s^{-1} and 15 s^{-1} (parallel reactions) respectively and Cc from B2 in ca. 2 s^{-1}. The *cis*−*trans* isomerization is much slower and thus a pseudo equilibrium involving AH^+, B2, B4, and Cc is established in the time scale of one second. In this pseudo equilibrium the major species are B2 and Cc, as shown in Scheme 1. From this stage the isomerization takes place and its rate is given by eq 7 where the term that multiplies k_i accounts for the mole fraction distribution of Cc at the pseudo equilibrium. Representation of the rate constant of the *cis*−*trans* isomerization rate constant as a function of pH should give a sigmoid curve with a plateau at higher pH values given by eq 8.[1]

$$k_{cis-trans} = \frac{K_h K_t}{[H^+] + K_h + K_h K_t} k_i + k_{-i} \quad (7)$$

$$k_{cis-trans}(\text{plateau}) = \frac{K_t}{1 + K_t} k_i + k_{-i} \quad (8)$$

In eq 8, the term that multiplies the rate constant k_i, represents the mole fraction of Cc when the pH is sufficiently high to convert all the flavylium cation into B2 and Cc at the pseudo equilibrium, as occurs at the plateau.

It is also important to retain the two limiting cases for the (slower) kinetics of the *trans*-chalcone appearance from flavylium cation upon pH jumps from very acid to the basic region: (i) in the case of hydration control a U shaped curve is obtained unless for higher pH values the *cis*−*trans* isomerization becomes slower (change of mechanism) leading to a plateau at higher pH values ; (ii) when the isomerization is the controlling step a sigmoid curve according to eq 7 and 8 is obtained, as observed for the flavylium cation itself.

When comparing the absorption spectra of the different species constituting the network, those of the flavylium cation and in less extent of the chalcones are red-shifted, while both B2 and B4 have absorption bands rather blue-shifted, due to the disruption of the $\pi-\pi^*$ conjugation in position 2 and 4 respectively.

The networks of flavylium compounds have been exploited as models for optical memories permitting to define *write*−*read*−*erase* cycles at the molecular level operated by light and pH inputs.[4] In particular when light is used as input the *trans*-chalcone is transformed into the *cis* form that spontaneously leads to the colorful flavylium cation. One interesting improvement is the possibility of enlarging the number of species in the network by designing a flavylium able to be reversibly converted in another flavylium, joining together two different reaction networks. This was partially achieved with 4′-acetamidoflavylium, which could be converted into the corresponding 4′-aminoflavylium upon hydrolysis under acidic conditions.[6] However, the system is irreversible. Here we report on a flavylium ester derivative that hydrolyzes to the corresponding acid under basic conditions and re-esterificates to the original compound in acidic conditions.

EXPERIMENTAL SECTION

General. All reagents and solvents were of analytical grade. ^1H and NMR spectra were recorded at 400 MHz respectively with a Bruker AMX400 in CD_3OD referenced to the solvent. Elemental analyses (EA) were performed in a Thermofinnigan Flash EA 112 series. Mass spectra

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were run on an Applied Biosystems Voyager-DE PRO. Spectroscopic experiments were carried out in buffered[7] water/ethanol 80:20 (v/v) solvent mixture. pH values were adjusted by the addition of a 0.1 M NaOH aqueous solution to a 0.1 M HCl aqueous solution, and measured with a MeterLab pHM240 pH meter from Radiometer Copenhagen. For solutions with HCl concentration above 0.1 M, pH was calculated from −log [HCl]. UV/vis absorption spectra were recorded with a Varian-Cary 100 Bio spectrophotometer or in a Shimadzu VC2501-PC. The stopped flow experiments were conducted in an Applied Photophysics SX20 stopped-flow spectrometer provided with a PDA.1/UV photodiode array detector.

Table 1. Crystal and Structure Refinement Data for Compound *trans*-Chalcone, Ct

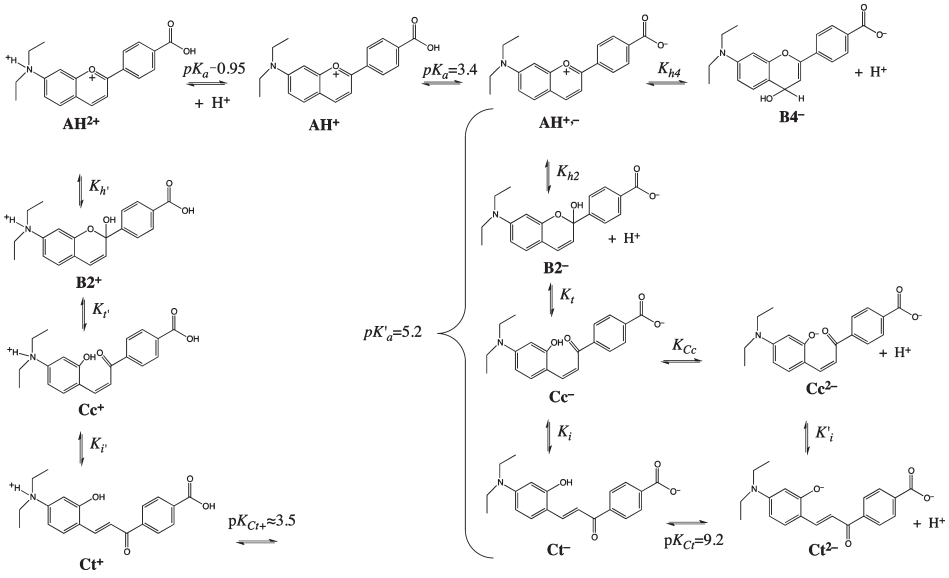
formula	$C_{21}H_{23}NO_4$
M_r	353.40
crystal description	orange plates
crystal size/mm	0.20 × 0.09 × 0.01
temperature/K	150(2)
crystal system	triclinic
space group	$P-1$
a /Å	12.039(2)
b /Å	13.110(2)
c /Å	13.346(2)
α /°	85.202(8)
β /°	67.787(7)
γ /°	68.725(8)
volume /Å^3	1813.7(5)
Z	4
$\rho_{calculated}$ /g cm^{-3}	1.294
$F(000)$	752
μ /mm^{-1}	0.089
θ range /°	3.61−23.11
index ranges	$-12 \le h \le 13$
	$-14 \le k \le 14$
	$-14 \le l \le 14$
reflections collected	19 454
independent reflections	4692 (R_{int} = 0.0829)
final R indices [$I > 2\sigma(I)$]	R_1 = 0.0506
	wR_2 = 0.0978
final R indices (all data)	R_1 = 0.1261
	wR_2 = 0.1307
largest diff peak and hole/e Å^3	0.206 and −0.212

Synthesis of 7-Diethylamino-2-(4-(methoxycarbonyl)-phenyl)-1-benzopyrylium Hydrogen Sulfate. The compound was prepared from condensation of 4-diethylamino-2-hydroxybenzaldehyde (0.31 g; 1.6 mmol) with 4-acetylbenzoic acid (0.26 g; 3 mmol).[8] The reagents were dissolved in 6 mL of acetic acid, 2 mL of H_2SO_4, and 2 mL of CH_3OH. The reaction mixture was stirred overnight. By the following day ethyl acetate was added and a purple solid precipitated, was filtered off and carefully washed with diethyl ether and dried yielding 0.58 g of 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium hydrogensulfate (1.4 mmol, 85%): ^{1}H NMR (CD_3OD, 300 K, 400.13 MHz), δ (ppm): 8.69 (1H, H4, d, ^3J = 7.8 Hz), 8.40 (2H, d, $H_{3'}$, $H_{5'}$, ^3J = 8.8 Hz); 8.27 (2H, d, $H_{2'}$, $H_{6'}$, ^3J = 8.7 Hz); 8.01 (2H, dd, H_3, H_5, ^3J = 8.7 Hz, ^4J = 5.9 Hz); 7.57 (1H, dd, H_6, ^3J = 9.5 Hz, ^4J = 2.4 Hz); 7.34 (1H, d, H8, ^4J = 1.7 Hz); 3.98 (3H, s, COO−CH_3); 3.83 (4H, q, N−CH_2), 1.39 (6H, t, CH_3). MALDI−TOF/MS: m/z (%): calcd for $C_{21}H_{22}NO_3$, 336.16; found, 336.16 [M^+] (100%). Anal. Calcd for $C_{21}H_{22}NO_3HSO_4$ (M_r = 433.47): C, 58.19; H, 5.35; N, 3.23. Found: C, 57.88; H, 5.60; N, 3.05.

Results for 7-Diethylamino-2-(4-(methoxycarbonyl)-phenyl)-1-benzopyrylium Chloride Obtained from Recrystallization of 2-(4-Carboxyphenyl)-7-diethylamino-1-benzopyrylium Hydrogen Sulfate in Acidic (HCl) Methanol. MALDI−TOF/MS: m/z (%): calcd for $C_{21}H_{22}NO_3^+$, 336.16; found, 336.2 [M^+] (100%). Anal. Calcd for $C_{21}H_{22}NO_3Cl\cdot 3H_2O$ (M_r = 418.70): C, 59.22; H, 6.53; N, 3.29. Found: C, 59.91; H, 6.46; N, 3.35.

Synthesis of 2-(4-Carboxyphenyl)-7-diethylamino-1-benzopyrylium Hydrogen Sulfate. This compound was prepared from condensation of 4-diethylamino-2-hydroxybenzaldehyde (0.58 g; 3 mmol) and 4-acetylbenzoic acid (0.49 g; 3 mmol). The reagents were dissolved in 9 mL of acetic acid and 3 mL of H_2SO_4. The reaction mixture was stirred overnight. By the following day, diethyl ether was added and a purple solid precipitated, was filtered off and carefully washed with

Scheme 3



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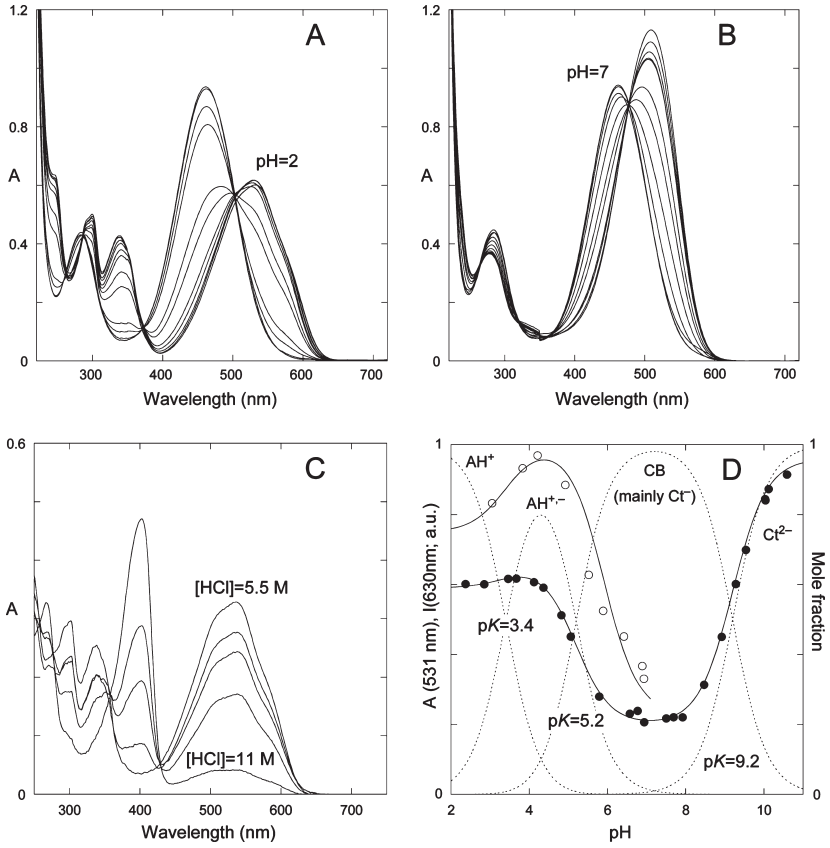

Figure 1. Spectral variations of the compound 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium, 3.43×10^{-5} M, in ethanolic (20%) aqueous solutions upon equilibration in the dark (3 h) as a function of pH: A, 2 < pH < 7; B, 7 < pH < 11.5; C, spectra at extremely acidic solutions; D, simultaneous fitting of the absorption at 531 nm (●) and emission at λ_{exc} = 500 nm, λ_{em} = 630 nm (○).

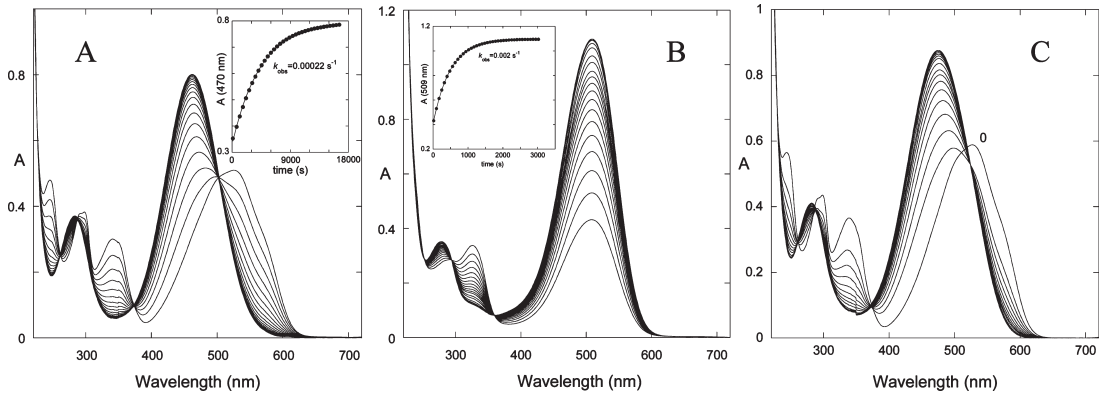

Figure 2. (A) Spectral variations of the compound 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium 3.4×10^{-5} M, upon a pH jump from 1.1 to 7.9. (B) The same for pH = 12.2. (C) The same for pH = 8.9.

diethyl ether and dried yielding 1.22 g of 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium hydrogen sulfate (2.9 mmol, 96%). ^{1}H NMR (CD_3OD/DCl, pD ≈ 1.0, 400.13 MHz), δ (ppm): 8.7 (1H, H4, d, $^3J_{H4-H3}$ = 7.8 Hz), 8.39 (2H, d, $H_{3'}$, $H_{5'}$, $^3J_{H3',H5'-H2',H6'}$ = 8.4 Hz); 8.22 (2H, d, $H_{2'}$, $H_{6'}$, $^3J_{H2',H6'-H3',H5'}$ = 8.4 Hz); 8.02 (2H, t, H_3, H_5, $^3J_{H3-H4}$ = 7.8 Hz, $^3J_{H5-H6}$ = 9.5 Hz); 7.55 (1H, dd, H_6, $^3J_{H6-H5}$ = 9.5 Hz, $^4J_{H6-H8}$ = 1.9 Hz); 7.33 (1H, d, H_8, $^4J_{H8-H6}$ = 1.5 Hz); 3.81 (4H, m, N−CH_2), 1.37 (6H, s, CH_3). MALDI−TOF/MS: m/z (%) calcd for $C_{20}H_{20}NO_3^+$, 322.14; found, 322.2 [M^+] (100%). Anal. Calcd for 0.9($C_{20}H_{20}NHO_3 \cdot 2HSO_4$) + 0.1($C_{20}H_{20}NO_3 \cdot HSO_4$)[9]: C, 47.31; H, 4.53; N, 2.76. Found: C, 47.54; H, 4.43; N, 2.88.

Isolation of the *trans*-Chalcone of 7-Diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium: (*E*)-1-(4-Acetylphenyl)-3-(4-diethylamino)-2-hydroxyphenyl)prop-2-en-1-one. The pH of 10 mL of ethanol/water (1:1) solution of 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium hydrogensulfate was adjusted to 7.0 with addition of 1 M NaOH dropwise. This mixture was warmed to reflux for 1 h and allowed to cool overnight. The solvent was decanted and the precipitated solid washed with ether several times and vacuum-dried to give a brown solid (25 mg, 18%).

^{1}H NMR (400.13 MHz, CD_3OD, room temperature, 400.13 MHz): δ (ppm) 8.13 (2H, d, H2′ + H6′ or H3′ + H5′, ^3J = 8.1 Hz), 8.08 (d, 1H,

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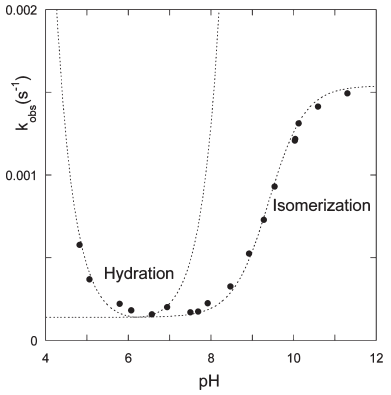


Figure 3. Representation of the rate constants of the direct pH jumps versus pH. Fitting was achieved according to eq 10 (hydration) and eq 11 (isomerization).

H_4, ^3J = 15.0 Hz), 8.03 (2H, d, H2′ + H6′ or H3′ + H5′, ^3J = 8.3 Hz), 7.49 (1H, d, H5, ^3J = 9.6 Hz), 7.48 (1H, d, H3, ^3J = 15.0 Hz), 6.31 (1H, dd, H6, ^3J = 8.9 Hz, ^4J = 1.5 Hz), 6.14 (1H, d, H8, ^4J = 2.0 Hz), 3.93 (3H, s, COO−CH_3), 3.41 (4H, q, N−CH_2), 1.19 ppm (6H, t, CH_3). MS (EI): *m/z* (%) calcd for MS (EI): *m/z* (%) calcd for $C_{21}H_{23}NO_4^{+}$, 353.16; found, 353.2 [$M^{+\bullet}$] (56%); 338.14 (100%) [M − $CH_3^{+\bullet}$]. Anal. Calcd for $C_{21}H_{23}NO_4 \cdot 1.5H_2O$ (M_r = 380.43): C, 66.30; H, 6.89; N, 3.68. Found: C, 66.23; H, 6.95; N, 3.68.

Ionized *trans*-Chalcone of 2-(4-Carboxyphenyl)-7-diethylamino-1-benzopyrylium Hydrogen Sulfate (Ct^{2-}). ^{1}H NMR (400.13 MHz, CD_3OD/NaOD, room temperature, 400.13 MHz): δ = 8.33 (1H, d, H4, ^3J = 14.9 Hz), 8.09 (2H, d, H2′, H6′ or H3′, H5′, ^3J = 8.4 Hz), 7.99 (2H, d, H2′, H6′ or H3′, H5′, ^3J = 8.4 Hz), 7.38 (1H, d, H5, ^3J = 9.0 Hz), 7.29 (1H, d, H3, ^3J = 14.9 Hz), 6.07 (1H, dd, H6, ^3J = 9.0 Hz, ^4J = 2.5 Hz), 5.94 (1H, d, H8, ^4J = 2.5 Hz), 3.54 (4H, q, N−CH_2), 3.35 (3H, s, CH_3OH, product of the ester hydrolysis), 1.18 ppm (6H, t, CH_3).

Single-Crystal X-ray Diffraction. Single crystals of the *trans*-chalcone, $C_{21}H_{23}NO_4$, of 7-diethylamino-2-(4-(methoxycarbonyl)-phenyl)-1-benzopyrylium were harvested from the crystallization vial and immersed in highly viscous FOMBLIN Y perfluoropolyether vacuum oil (LVAC 140/13). A suitable crystal was selected and mounted on a Hampton Research CryoLoop with the assistance of a Stemi 2000 stereomicroscope.[10] Data were collected on a Bruker X8 Kappa APEX II charge-coupled device (CCD) area-detector diffractometer (Mo Kα graphite-monochromated radiation, λ = 0.71073 Å) controlled by the APEX2 software package,[11] and equipped with an Oxford Cryosystems Series 700 cryostream monitored remotely using the software interface Cryopad.[12] Images were processed using the software package SAINT+,[13] and data were corrected for absorption by the multiscan semiempirical method implemented in SADABS.[14] The structure was solved by direct methods implemented in SHELXS-97,[15,16] and refined from successive full-matrix least-squares cycles on *F2* using SHELXL-97.[15,17] All non-hydrogen atoms were successfully refined using anisotropic displacement parameters.

Hydrogen atoms associated with the hydroxyl (O−H) groups were markedly visible in difference Fourier maps and were included in the structure with the O−H distances restrained to 0.90(2) Å, and U_{iso} fixed at 1.5 × U_{eq} of the parent oxygen atom. H atoms bound to carbon were located at their idealized positions using appropriate *HFIX* instructions in SHELXL: 43 for the aromatic, 23 for the −CH_2 carbons and 137 for the terminal −CH_3 methyl groups. All these atoms were included in subsequent refinement cycles in riding-motion approximation with isotropic thermal displacements parameters (U_{iso}) fixed at 1.2 or 1.5 × U_{eq} of the carbon atom to which they are attached.

Information concerning the crystallographic data collection and structure refinement are summarized in Table 1. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC-838069. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax, +44-(0)1223−336033, or e-mail, deposit@ccdc.cam.ac.uk).

RESULTS AND DISCUSSION

2-(4-Carboxyphenyl)-7-diethylamino-1-benzopyrylium Hydrogen Sulfate. The pH dependent spectral variations of equilibrated solutions of the compound 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium, Scheme 3, are represented in Figure 1.

The data are compatible with a sequence of reactions as shown in Scheme 3. The small spectral variations of the absorption regarding the first inflection (pK = 3.4) are attributed to the deprotonation of the carboxylic moiety, parts A and D of Figure 1. The deprotonation of the carboxylic substituent is better detected by fluorescence, Figure 1D (open circles), as previously reported for the analogous compound 4-(2-carboxyphenyl)-7-diethylamino-4′-dimethylamino-1-benzopyrylium.[18] The higher acidity of the carboxylic substituent, pK_a = 3.4, in comparison with benzoic acid pK_a = 4.2, is expected from the influence of the positive charge of the flavylium cation.

The network of chemical reactions characteristic of the flavylium system, eq 5, takes place at higher pH values, pK'_a = 5.2, and by consequence all the species involved are deprotonated at the carboxylic moiety, in particular the flavylium cation in this pH range can be considered as having a zwitterionic nature. The major species of CB in eq 5 is the ionized *trans*-chalcone, Ct^-, based on the shape and position of the absorption spectrum and on ^{1}H NMR data, see below. Finally in Figure 1B the ionized *trans*-chalcone (Ct^{2-}) is formed from the *trans*-chalcone (Ct^-). At extremely acidic pH values the amine protonates with p$K_a \approx$ −0.95 (Figure 1C).

From the data of Figure 1, eq 9 can be written

$$K'_a = K_{h4} + K_{h2} + K_{h2}K_t + K_{h2}K_tK_i = 10^{-5.2} \quad (9)$$

The formation of (ionized) *trans*-chalcone (Ct^{2-}) at pH = 12 was confirmed by ^{1}H NMR on the basis of the large coupling constant between H3 and H4, Figure 7B. Titration of this species followed by UV−vis back to acid up to pH = 7 reproduces the data from Figure 1B.

In order to characterize the kinetics of the network of chemical reactions, a series of pH jumps from equilibrated solutions at pH = 1.1 to higher pH values was performed, Figure 2. If the pH jump is carried out to pH = 7.9, Figure 2A, the flavylium cation (ionized on the carboxylic group) is the species observed immediately after the jump and the system evolves to the *trans*-chalcone (also ionized in the carboxylic group). At pH = 8.9, Figure 2C, the first absorption spectrum after the pH jump indicates the presence of the flavylium cation that evolves over time to ionized *trans*-chalcone through three kinetic processes, the first one much faster (see below stopped flow experiments). In the case of the same experiment to pH = 12.2, Figure 2B, the species immediately formed after the jump is not the flavylium cation, but instead a species with an absorption band centered at 509 nm appears. This behavior is an indication that hydration of

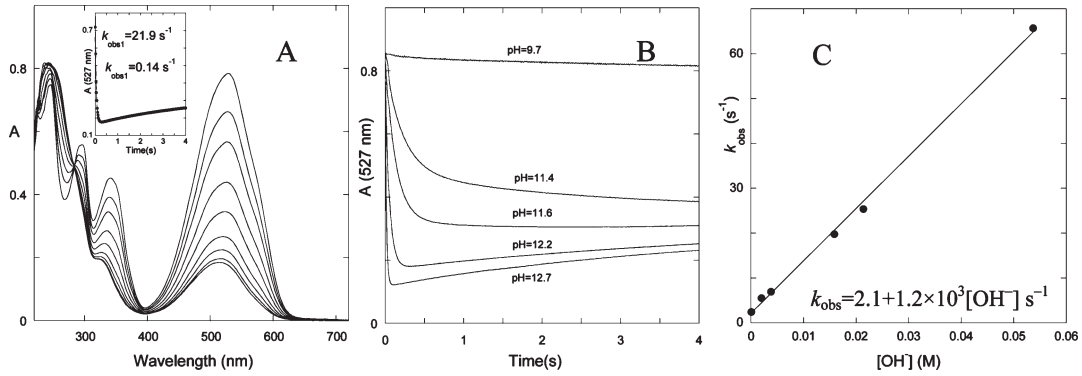


Figure 4. (A) Spectral variations of the compound 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium 4.3 × 10^{-5} M, after a pH jump from 1.1 to 12.2 followed by stopped flow: (B) stopped flow traces after a pH jump from 1.1 to the basic region; (C) rate constants as a function of the hydroxyl concentration.

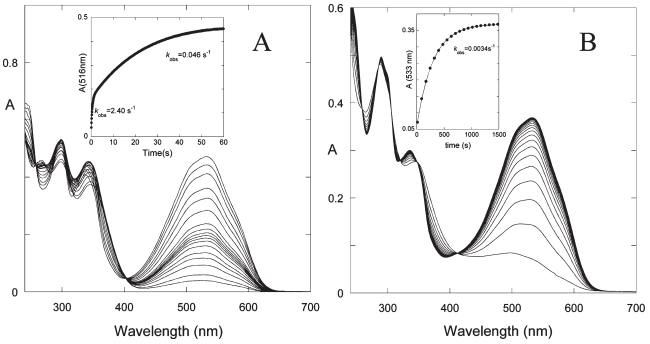
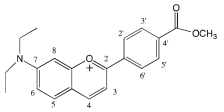


Figure 5. (A) Spectral modifications of the compound 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium 4.3 × 10^{-5} M in water−ethanol (20%) upon a reverse pH jump from solutions at pH = 13 (nonequilibrated) followed by stopped flow. (B) The same upon equilibration followed by a normal spectrophotometer.

Scheme 4



the flavylium cation takes place at this pH value before the time needed to record an absorption spectrum in our spectrophotometer (ca. 1 min). The nature of the species formed as the result of this hydration will be discussed below on the basis of the stopped flow measurements.

More insight on the system was obtained by representing the rate constants of the direct pH jump experiments like those reported in Figure 2, as a function of pH, Figure 3. At lower pH values the fitting follows eq 10, and we can attribute this process to an hydration control (see below in the stopped flow section the details for the calculation of the [OH$^-$] coefficient). At higher pH values the hydroxyl attacks the flavylium cation and hydration becomes faster than the isomerization rendering this last process as the rate-determining step. The plateau at higher pH values is reached when the ionized- Cc^{2-} attains the pseudo equilibrium, i.e., an equilibrium of all the basic species before significant formation of the Ct^{2-}. The fitting achieved by means of eq 11 reflects the fraction of Cc^{2-} existent at the pseudo equilibrium.

$$k_{hydration}\ (s^{-1}) = 1.4 \times 10^{-4} + 35[H^+] + 1.2 \times 10^3[OH^-] \qquad K_{h2} = 4 \times 10^{-6}\ M^{-1} \tag{10}$$

$$k_{isomerization}(s^{-1}) = \frac{10^{-9.4}}{[H^+] + 10^{-9.4}} \times 0.0014 + 1.4 \times 10^{-4} \tag{11}$$

The concept of pseudoequilibrium presented in eq 6 can be extended to the basic region, by adding eq 12, considering that in eq 6 the species are ionized at the carboxylic substituent.

$$\begin{aligned} &AH^{+,-} + H_2O \rightleftharpoons CB\hat{} + H_3O^+ \qquad K\hat{}_a \\ &[CB\hat{}] = [B4^-] + [B2^-] + [Cc^-] \\ &Cc^- \rightleftharpoons Cc^{2-} + H^+ \qquad K_{Cc^-/Cc^{2-}} \end{aligned} \tag{12}$$

The mole fraction of Cc^{2-} being given by eq 13

$$\chi_{Cc^{2-}} = \frac{K_{h2}K_tK_{Cc^-/Cc^{2-}}}{[H^+]^2 + K\hat{}_a[H^+] + K_{h2}K_tK_{Cc^-/Cc^{2-}}} \tag{13}$$

According to the fitting of eq 11, $((K_{h2}K_tK_{Cc^-/Cc^{2-}})/(K\hat{}_a)) = 10^{-9.4}$ and the intrinsic value of the isomerization rate constant is $k_i' = 0.0014\ s^{-1}$. Considering that the experimental evidence points out to $[CB\hat{}] \approx [Cc^-]$ and $K\hat{}_a \approx K_{h2}K_t$ it can be concluded that $K_{Cc^-/Cc^{2-}} \approx 10^{-9.4}$. This value is close to $K_{Ct^-/Ct^{2-}} = 10^{-9.2}$, Figure 1D, as expected from the usually observed similarity between the acidity constants of the *cis*- and *trans*-chalcones.[19]

In order to characterize the pseudoequilibrium, a series of pH jumps from pH = 1.0 monitored by stopped flow was carried out, Figure 4.

The stopped flow data shows that, besides the slow process shown in Figure 2B attributed to the *cis*−*trans* isomerization (time scale of hours), two other previous kinetic processes are detected, Figure 4. At basic medium (pH = 12.2) a very fast process with rate constant 21.9 s^{-1}, corresponds to the disappearance of the flavylium cation, i.e., its hydration. This is followed by a second process leading to a raise of the absorption in the visible. These results can be explained if during the first step $B4^-$, $B2^-$, and Cc^{2-} are formed and in a second step the kinetic product $B4^-$ gives rise to the final pseudoequilibrium involving essentially Cc^{2-}. This corresponds to the pseudo-equilibrium above-reported, the state from which the final

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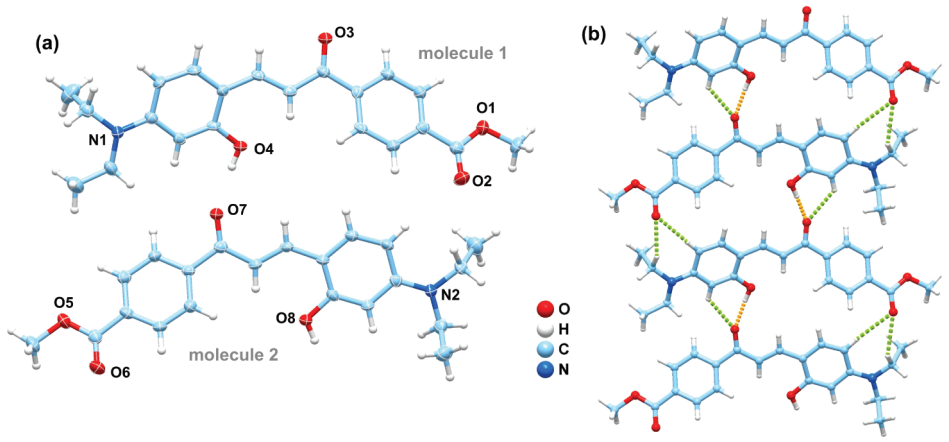

Figure 6. (a) Asymmetric unit cell of the crystalline structure of the *trans*-chalcone of 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium showing the labeling scheme for the oxygen and nitrogen atoms; All atoms are represented as thermal ellipsoids drawn at the 50% probability level, excluding the hydrogen atoms which are drawn as spheres with arbitrary radii. (b) Strong O−H···O (orange dashed lines) and weak C−H···O (green dashed lines) hydrogen bounds between adjacent molecules.

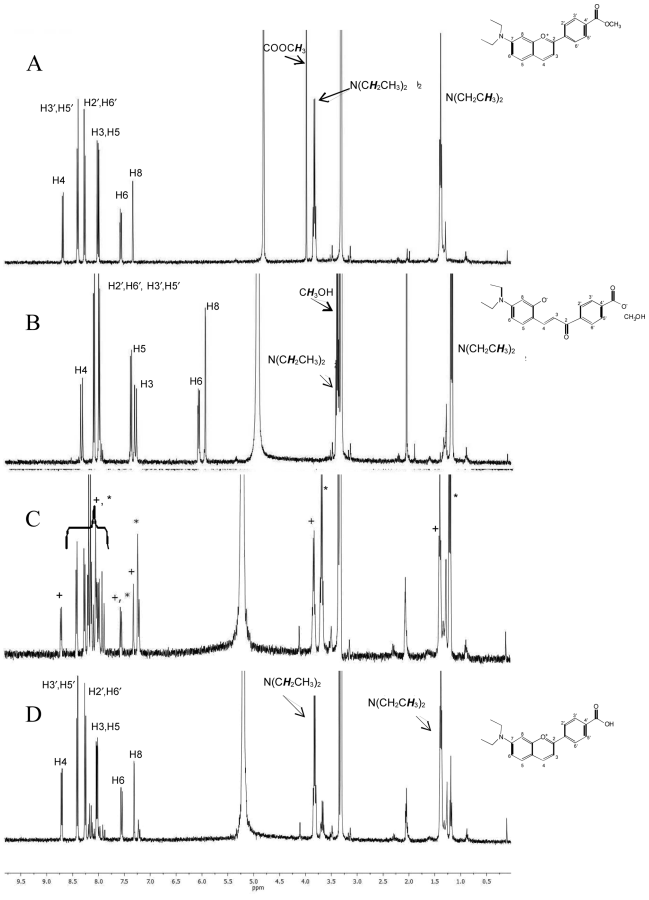

Figure 7. ^{1}H NMR spectra at room temperature in CD_3OD: (A) 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium; (B) the same compound after addition of NaOD (Ct^{2-} form); (C and D) evolution of the system after addition of DCl to the previous solution (+ denotes the presence of the flavylium cation and ∗ the presence of the Ct form).

step occurs. The existence of a very fast tautomeric equilibrium (between $B2^-$ and Cc^-) is expected from the previously described catalytic effect of the hydroxyl ion in the ring-opening closure.[1] The faster process is proportional to the hydroxyl ion concentration, Figure 4C, with an observed rate constant 1.2×10^3 s^{-1} and can be attributed to the sum of the rate constants $k_{B2}^{OH} + k_{B4}^{OH}$, responsible for the hydroxyl ion attack to the flavylium cation in positions 2 and 4, respectively.

A sequence of pH jumps from equilibrated solutions at pH = 1 to 13 and immediately back to pH = 1 have been performed, the absorption being monitored in the last step, Figure 5A. Protonation is faster than the mixing time of the stopped flow and by consequence all the species present at pH = 13 are immediately frozen in the respective protonated species. In other words, all $B2^-$, $B4^-$ and Cc^{2-} existent at pH = 13 upon the initial pH jump become B2, B4, and Cc, the observed kinetic processes regard these species.

The biexponential trace in the inset of Figure 5A is in accordance to a faster kinetics that converts B2 and B4 into flavylium cation (2.4 s^{-1}), followed by a slower one leading to more flavylium cation from Cc via B2. On the other hand, the conversion of Ct to flavylium cation is much slower as shown in Figure 5B. The ratio of the amplitudes in Figure 5A is 0.38 meaning that the pseudo equilibrium at pH = 13 was constituted by 38% of $B2^-$ and $B4^-$ and 62% of Cc^{2-}.

7-Diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium Hydrogen Sulfate. The compound 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium, Scheme 4, can be obtained according to the synthetic procedure described in the experimental part or from the previously studied 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium in acidic methanol, see below. For this reason the following experiments have been carried out in water containing 20% of ethanol. In this case the solutions are stable, unless differently is mentioned.

Crystal Structure of the *trans*-Chalcone. A crystalline material of *trans*-chalcone of 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium suitable for single-crystal X-ray diffraction analysis was obtained by recrystallization in ethanol of the precipitate obtained in a mixture water:ethanol, and the unveiled structure confirms undoubtedly the formation of the chalcone species in the configuration *trans* (Figure 6a). Systematic searches in the literature and in the Cambridge Structure Database (CSD, Version 5.32 of 2011)[20,21] confirm that this compound was not reported previously. The crystal structure was determined in the triclinic system and in the centrosymmetric space group *P*−1

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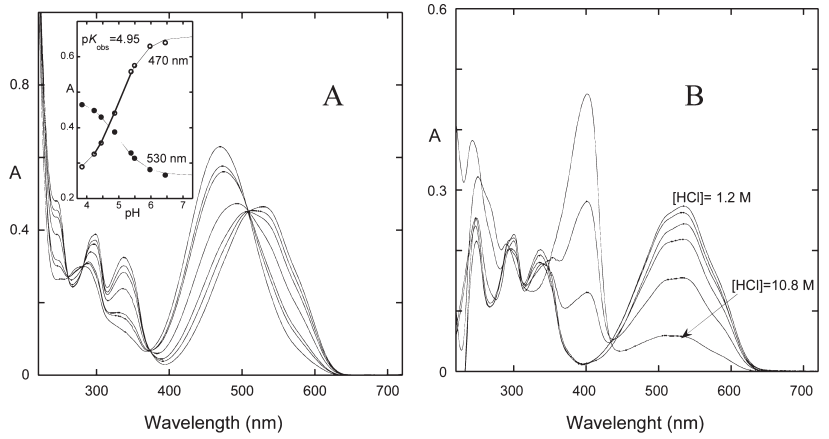


Figure 8. Absorption spectra of the compound 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium, 2.7×10^{-5} M in water−ethanol (80:20) upon equilibration: (A) $-3.9 < pH < 7.9$, fitting was achieved for $pK'_a = 4.95$; (B) at extremely acidic medium (1.1×10^{-5} M, in water), inflection point occurs for [HCl] ≈ 9 M.

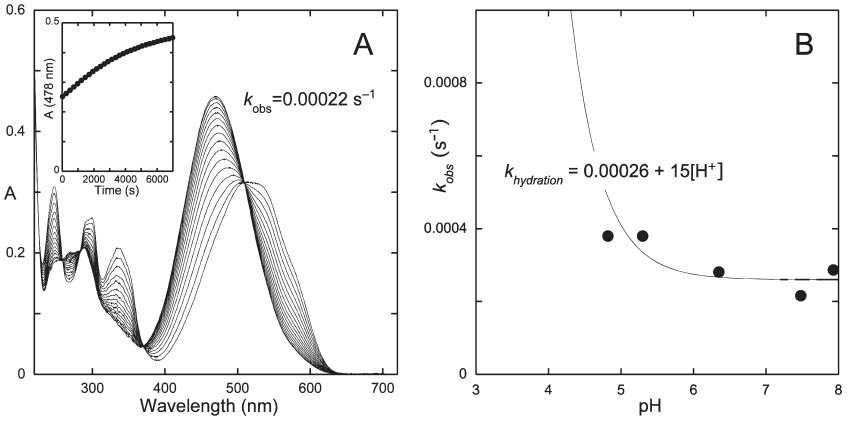
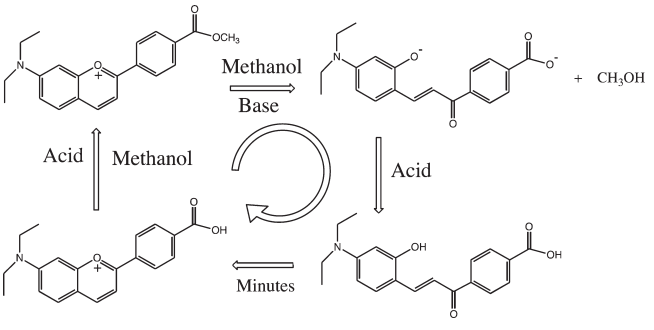


Figure 9. (A) Spectral variations after a pH jump from equilibrated solutions of the compound 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium in water−ethanol (80:20) at pH = 1 to pH = 7.5 (1.8×10^{-5} M); (B) Observed rate constants as a function of pH upon direct pH jumps from pH = 1.0.

Scheme 5



(more details related with the crystallographic data collection and structure refinement can be found in the Experimental Section and Table 1). The asymmetric unit cell (asu) consists of two crystallographic independent Ct molecules, each one rotated 180° in the horizontal plane relatively to the other. Furthermore, the two molecules display distortion conformational considerably distinct. In molecule 1 (that contains the N1 atom) the dihedral angle between the average planes of the two aromatic rings is c.a. 35.16°, while the correspondent angle in the molecule 2 (that contains the N2 atom) is c.a. 31.87°. Adjacent Ct molecules interact through strong O−H···O intermolecular hydrogen bonds (orange dashed lines in Figure 6b) involving one hydroxyl group and oxygen atom of the carbonyl group (C═O) in the neighboring molecule leading to the formation of one-dimensional supramolecular arrangement: O4−H1···O7 with $d_{(H1\cdots O7)}$ = 1.858(19) Å and angle$_{(O4-H1-O7)}$ = 171(4)°, and O2−H2···O3 with $d_{(H2\cdots O3)}$ = 1.814(19) Å and angle$_{(O8-H2-O3)}$ = 177(3)°; symmetry transformations used to generate equivalent atoms, (i) *x*, *y*−1, *z*. This supramolecular arrangement is further reinforced by the cooperative effect of various intermolecular C−H···O weak interactions (green dashed lines in Figure 6b): C14−H14···O6, C16−H16···O7, C18−H18A···O6, C35−H35···O2, C37−H37···O3, C39−H39B···O2, with H···O distances in the range between 2.558(3) and 2.716(3) Å and the C−H−O angles ranging from 131.3(2) and 177,6(2)°.

De-Esterification of 7-Diethylamino-2-(4-(methoxycarbonyl)-phenyl)-1-benzopyrylium. When aqueous solutions of the compound 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium in *d*-methanol are basified the ^{1}H NMR spectrum taken

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immediately after shows unequivocally the disappearance of the singlet of the methoxy group and the appearance at 3.34 ppm of a singlet that is characteristic of (nondeuterated) methanol. Acidification of this solution shows the appearance of the protonated *trans*-chalcone that evolves to 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium, Figure 7.

The spectral variations of equilibrated solutions of the compound 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium, are shown in Figure 8. The study was carried out in acidic-neutral medium in order to avoid the formation of 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium.

The absorption spectra presented in Figure 8A shows a single set of isosbestic points corresponding to the equilibrium between flavylium cation and *trans*-chalcone. This behavior is very similar to the parent compound 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium and a comparable global acidity constant K'_a is obtained. The spectra at lower pH values are also similar to those of the free carboxylic acid, showing a $pK_a \approx -0.95$.

Solutions of the compound 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium at pH = 1.0 were submitted to pH jumps to higher pH values and the UV−vis absorption modifications monitored, Figure 9.

The initial spectrum in Figure 9A is the flavylium cation that evolves to the *trans*-chalcone, an identical pattern being observed in the pH range between 3 and 8. This behavior indicates a process controlled by the hydration reaction similarly to the carboxyl derivative. Fitting was achieved for $k_{h2} = 0.00026\ s^{-1}$ and $k_{-h2} = 15\ M^{-1}\ s^{-1}$, $pK_h = 4.8$, that compares with 5.4 for the carboxylic derivative showing that the ester compound is more easily hydrated.

Solutions of the compound 2-(4-carboxyphenyl)-7-diethylamino-1-benzopyrylium in methanol are converted into the methyl ester derivative 7-diethylamino-2-(4-(methoxycarbonyl)phenyl)-1-benzopyrylium upon addition of hydrochloric acid. This fact allows to conceive an unidirectional cycle (Scheme 5) where the flavylium ester derivative is converted into the free carboxylic acid through the respective *trans*-chalcone upon alternating acid−base inputs. While the base catalyzed hydrolysis of esters is a well-known reaction, the esterification of a carboxylic cid at room temperature is less usual. In the case of the present compound the positive charge of the benzopyrylium unit facilitates the attack of methanol to the carboxylic acid group on the *para* position.

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NOTE ADDED AFTER ASAP PUBLICATION

This article posted ASAP on December 28, 2011. Equation 6 has been revised. The correct version posted on January 3, 2012.