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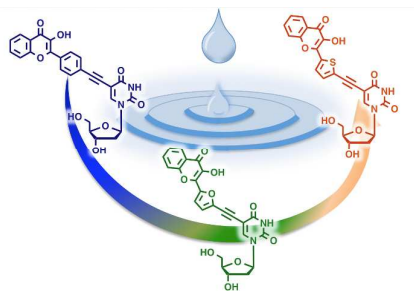
We designed and developed fluorescent deoxyuridine analogues with strong sensitivity to hydration for major groove labelling of DNA

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ARTICLE

Development of environmentally sensitive fluorescent and dual emissive deoxyuridine analogues

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Ratiometric and environment-sensitive fluorescent dyes present attractive advantages for sensing interactions in DNA research. Here, we report the rational design, synthesis, and photophysical characterization of 2-thienyl-, 2-furyl- and 2-phenyl-3-hydroxychromones bonded to the C-5 position of deoxyuridine. Since these two-color nucleosides were designed for incorporation into ODNs, we also investigated the sensitivity of the ratiometric response to hydration by using acetonitrile/water mixtures and neat solvents. The synthesized 2-thienyl and 2-furyl conjugates were found to exhibit more red-shifted absorption (by 31-36 nm) and emission (by 77-81 nm of N* band), two-fold increased molar absorption coefficients, and dramatically enhanced (by 3-4.5 times) fluorescence quantum yields. Demonstrating one manifold increase in brightness, they preserve the ability of exquisite ratiometric responses to solvent polarity and hydration. This makes the new fluorescent nucleoside analogues highly relevant for subsequent labeling of the major groove in nucleic acids and sensing their interactions.

Introduction

Fluorescence spectroscopy is a highly versatile tool widely applied in biomolecular researches.[1] Unfortunately, the very low intrinsic fluorescence of DNA allows only a very limited number of applications by this technique.[2] In contrast, the introduction of fluorescent probes provides a broad access to a variety of applications that include probing DNA hybridization,[3] typing single-nucleotide polymorphism (SNP),[4] and monitoring the dynamics of DNA/protein complexes,[5,6] to name a few. One major approach to design a DNA-fluorescent sensor is to incorporate into DNA, a fluorescent nucleoside analogue as a fluorescent signal transducer.[7] Fluorophores that exhibit extreme sensitivity to environmental changes and interactions are highly desirable to provide site-specific responses in sensing.

A large number of analogues of fluorescent nucleosides, such as the widely used 2-amino-purine label (2-AP), responds to environmental changes by a variation in the intensity of fluorescence.[8] However, low sensitivity to the surrounding environment and low quantum yields in DNA duplexes limit the use of such intensiometric probes. These limitations encourage investigating the synthesis of advanced emissive nucleosides based on new mechanisms of response. Ratiometric sensing, also referred to as λ-ratiometric,[9] is one such approach, and is based on recording a ratio of the intensities at two or more wavelengths. Compared to simple intensity sensing, ratiometric sensing is advantageous since it compensates for instrumental factors such as fluctuations in the intensity of the light source, and because the resulting intrinsically calibrated

analytical response does not depend on the concentration of the applied dye.[10] To this point, two-channel probing was mainly based on double-labeling of nucleic acids as for the construction of fluorescence resonance energy transfer (FRET) and excimer pairs.[4e-f,5j,6c] Difficulties and cost of synthesis obviously represent limitations for this approach. Alternatively, λ-ratiometric sensing can be performed with a single fluorophore[9] using environment sensitive probes. One class of these probes comprises single-band solvatochromic dyes, which respond by a spectral shift due to an excited-state intramolecular charge transfer (ICT). However, these single λ-ratiometric emitters remain faintly explored in the field of DNA sensing[11] mainly due to the strong variations of the intensity of fluorescence that accompany the shifts. Dual emissive fluorophores emerge as a second class. These dyes exhibit two emission bands as a result of twisted excited-state intramolecular charge transfer (TICT)[12] or excited-state intramolecular proton transfer (ESIPT).[13] Dual emissive dyes are very attractive because they offer facile and straightforward quantification through the ratio of their two bands. Nevertheless, they are still poorly exploited because of the very limited number of dyes that display such properties and the difficulties in their syntheses.

Among fluorophores with dual emission, 3-hydroxychromones (3HCs) appear particularly promising in terms of photophysics and applications as biosensor units. Due to the ESIPT reaction, 3HCs exhibit two excited states, the normal form (N*) and the tautomer form (T*), that provide two well separated emission bands (Fig. 1).[14] 3HC fluorophores offer exceptional solvatochromism,[15] since both an increase in

the hydrogen bond donor strength and the dielectric constant of solvents inhibit the ESIPT reaction and thus, decrease the relative intensity of the T* band.[16] Therefore, changes in the polarity and the hydration of the microenvironment of 3HCs can be monitored by measuring the ratio of the intensities of the two emission bands (I_{N*}/I_{T*}).[15] This unique behaviour of 3HCs was the cornerstone for the design of environment-sensitive fluorescent probes for a large range of applications. These include the characterization of supercritical fluids,[17] micelles,[18] phospholipids bilayers,[19] plasma membranes of living cells,[20] and biomolecular interactions.[21,22] Their applications as fluorescent nucleoside analogues to label oligonucleotides (ODNs) has only been recently explored.[23,24]
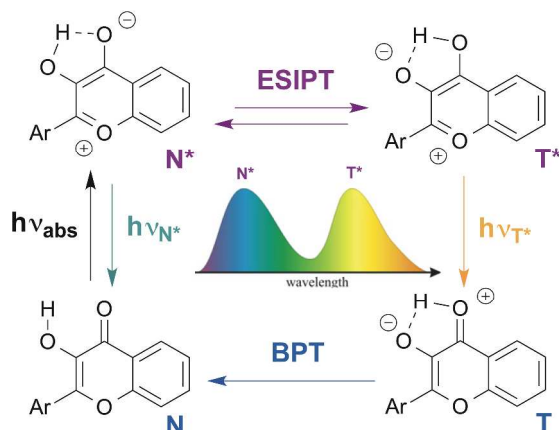

Fig. 1 Excited State Intramolecular Proton Transfer (ESIPT) reaction of 3-hydroxychromones: genesis of the dual emission. BPT: Back Proton Transfer.

The fluorescent C-nucleoside **1** using 2-thienyl-3-hydroxychromone moiety was first designed as a substitute for natural bases for internal labeling of ODNs and sensing (Fig. 2).[25,26] As a next step, external labeling of DNA major groove was addressed by compiling 2-thienyl-3-hydroxychromone and uracil at the 5-position via an ethynyl bond.[26] The synthesized conjugate demonstrated that the nucleobase and 3HC fragments were coupled into an electronic conjugated system, which attained strong ICT character upon excitation.
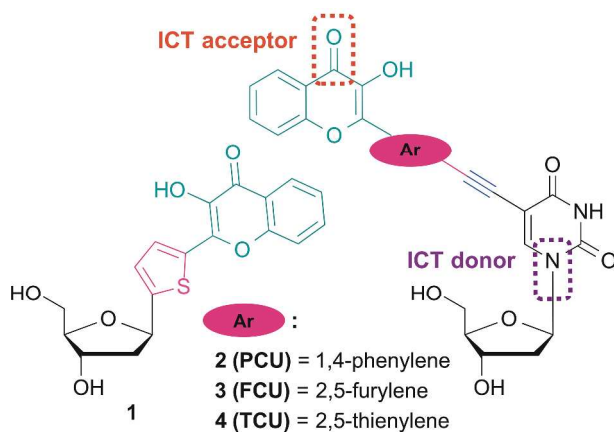

Fig. 2 Fluorescent nucleoside analogues incorporating 3-hydroxychromone fluorophore as a nucleobase surrogate **1**[23,24] and natural base modifier **2-4**.[25]

The ICT states are crucial in achieving the solvent-dependent two-band emission of 3HC dyes. The substitutions in the position 2 of the chromone demonstrates the strongest modulating power[27a] that allows reaching a dynamic equilibrium between the populations of ICT and ESIPT states, from which the dual emission originates.[27b] With the aim to label DNA and to tune the spectroscopic properties of the conjugates for sensing, reliable synthesis of 2'-deoxyuridine conjugates bearing aryl groups of different natures connected to the chromone was developed (Fig. 2). Herein, we report the synthesis and the photophysics of 2'-deoxyuridine coupled to 2-phenyl-, 2-furyl- and 2-thienyl-3-hydroxychromone referred as **PCU**, **FCU** and **TCU**, respectively (**2-4**).

Results and discussion

The 3HC deoxyuridines **PCU**, **FCU**, and **TCU** were furnished by a convergent synthetic strategy based on Sonogashira couplings and Algar-Flyn-Oyamada reaction (Scheme 1). Sonogashira reaction[28] is widely applied to couple aromatic scaffolds, including dyes such as pyrenes,[12b,29] fluorenes,[30] naphtalenes and anthracenes,[31] to deoxyuridine. This palladium-catalyzed cross-coupling proceeds usually by assembling a C-5 iodinated uracil moiety with an aromatic alkyne.[32] Therefore, the ethynyl moiety is generally introduced to the aromatic molecule through a cross-coupling with TMS-acetylene, after which the alkyne is deprotected from its silyl group and directly coupled to the heterocyclic 5-iodo-2'-deoxyuridine derivative.

We applied this approach for the preparation of the target compounds. However, we faced difficulties in the elaboration of ethynylchromones (Scheme S1), and found that the Sonogashira coupling between our tested ethynylchromone and 5-iodo-2'-deoxyuridine was sluggish and low yielding. This might be attributed to a competition between homo- and cross-couplings. Indeed, it has been known that homo-coupling is favored in the presence of electron-withdrawing groups on aryl alkynes.[28] So the 3HC substitution brings electron deficiency to the terminal alkyne, consequently increasing the competition of the homo- and cross-couplings to access the catalyst. To overcome these difficulties, we adopted an alternative approach based on reacting the electron-rich ethynyl-deoxyuridine derivative with the electron-poor bromo-arylchromones. The new suggested strategy established a more reliable synthetic access to the target compounds.
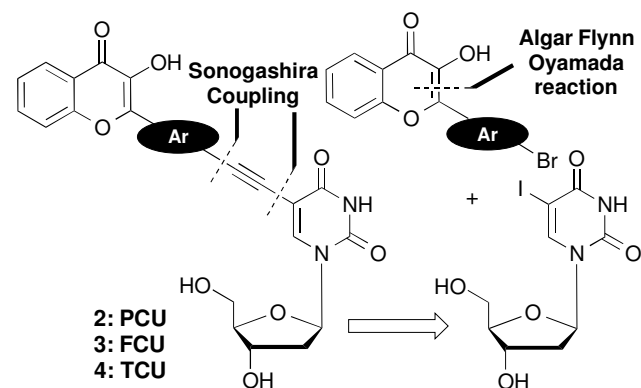
Scheme 1. Retrosynthetic analysis of the targeted two-color emission nucleosides **2-4**.

The preparation of the 3HC key intermediates **9–15** required for the subsequent coupling with the 5-ethynyldeoxyuridine

derivative is described in Scheme 2. The bromochromones **9–11** were obtained using the fast and versatile Algar-Flynn-Oyamada reaction.[33] Thus, starting from 2-hydroxy-acetophenone **5**, an aldol condensation/dehydratation process with aldehydes **6-8** in the presence of sodium hydroxide in ethanol followed by an oxidative cyclization with aqueous hydrogen peroxide provided the chromones **9–11** (48-58 %).
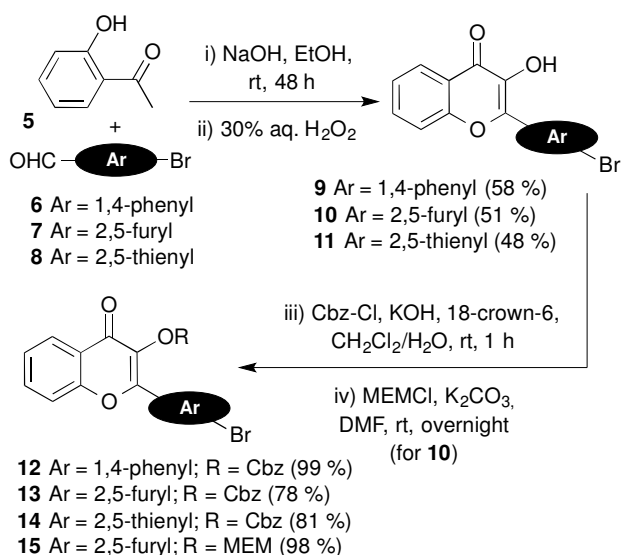

Scheme 2. Synthetic preparation of the fluorescent chromone-coupling partners.

The synthesis of the chromone-deoxyuridine conjugates required masking the reactive 3-OH group of the chromone. The benzyloxycarbonyl group (Cbz), which was successfully employed during the synthesis of 3HC-containing C-nucleosides and ODNs,[24] and the more robust methoxyethoxymethyl group (MEM)[25] were chosen as the protecting groups. The Cbz protection of the 3-OH group of chromones **9-11** was performed under phase-transfer catalysis conditions,[34] delivering compounds **12-14** in good yields. Protection with the MEM group was obtained by treatment of the furyl chromone **10** with potassium carbonate and MEMCl in DMF, producing compound **15**.

Next, we synthesized the 5-ethynyldeoxyuridine partner **18**, using common procedures (Scheme 3). The protection of deoxyuridine **16** before the iodination of the nucleobase at the C-5 position with cerium(IV) ammonium nitrate turned out be more convenient and efficient.[35] Sonogashira coupling of the iodo intermediate **17** with TMS-acetylene followed by treatment with tetrabutylammonium fluoride furnished the terminal alkyne **18** in satisfactory yields (64% in 2 steps).[†]

Preliminary attempts of the final assembly were performed with the protected bromochromone **15** (Scheme 3). Different conditions were examined including the catalyst (Pd(PPh$_3$)$_4$ or PdCl$_2$(PPh$_3$)$_2$), the loading (5-20 mol%), and the solvent (toluene, dioxane, THF and DMF). All screenings converged to two major products: the desired coupling derivative **19** and the 5-*endo-dig* cyclization side-product **20**. Despite the use of DMF, which is reported to minimize the formation of the bicyclic furanopyrimidine,[36] the formation of the *endo* product could not be avoided. The ethynyl-coupled product **19** was difficult to purify and thus, was obtained in 30 % yield after silica gel and reverse phase chromatographies. NMR and MS

analysis,[††] and chemical means supported the proposed structure of the *endo* product. As reported amongst others[37] by McGuigan et al.,[38-40] after the Sonogashira coupling, an extended treatment with CuI/Et$_3$N in THF or MeOH at reflux for several hours enabled us to fully convert the coupled alkynyl derivative **17** into the 5-*endo* cyclized side product **18** (Scheme 3).
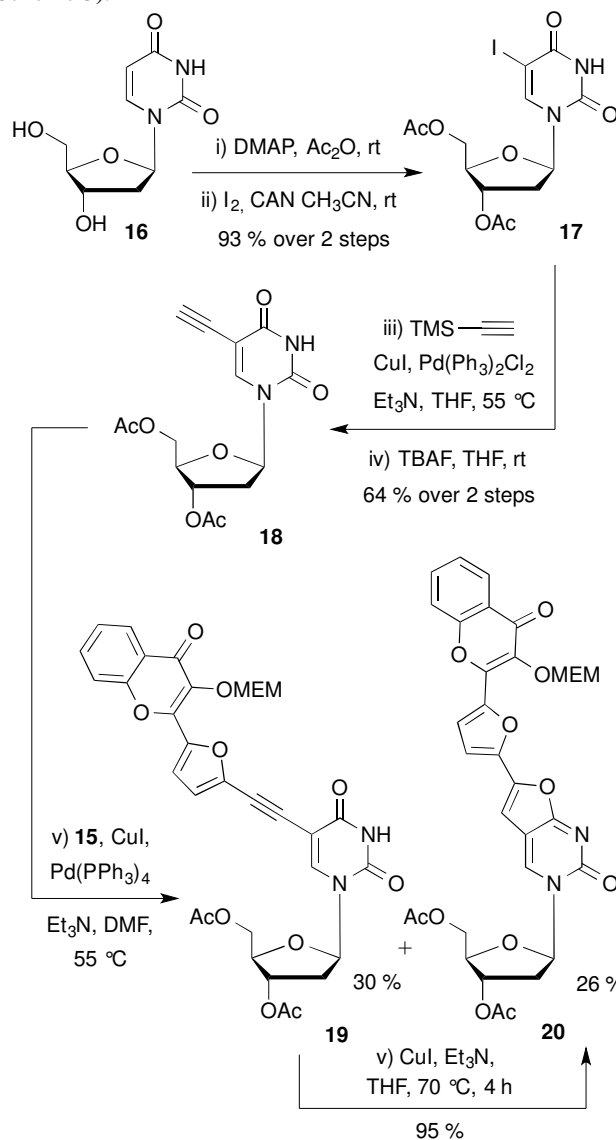
Scheme 3. Preliminary Sonogashira couplings on substrate **18** having no N^3-protecting group on the uracil moiety.

Endo-cyclisation of 5-ethynyluridine derivatives is a well-known reaction that is favored by prolonged reaction time at elevated temperatures, high CuI loading, and the presence of electron-withdrawing groups on the coupling partner.[41] Although the copper-free Sonogashira coupling was widely reported in the last decade,[42] no product at all was formed when copper-free conditions were applied to the reaction of acetylenic and iodo partners as evidenced by TLC. Protection of the N^3-imide with the base labile benzoyl group is known to prevent 5-*endo* cyclisation.[43] Consequently, we selected this type of protection, using *p*-toluoyl rather than benzoyl, since the former presents the advantage of displaying a characteristic

methyl singlet easy to detect by NMR. To set up the imide protecting group, mild conditions (*p*-toloyl chloride, Et$_3$N in pyridine) were employed to provide **21** (82%) as the building block for the final assembly of the two fragments (Scheme 4). Second Sonogashira coupling was then efficiently used for that purpose. Thus, when using a standard catalytic system (PdCl$_2$(Ph$_3$)$_2$, CuI, Et$_3$N in THF), the reaction proceeded cleanly to give the desired coupled derivatives **22-24**. It is noteworthy that the yields of C(sp)-C(sp^2) cross coupling for the five-membered ring partners **23** and **24** (82 and 87%) are more satisfactory as compared to the one obtained with the phenyl moiety **22** (60%). The lower yield of the phenyl derivative could be attributed to the higher steric hindrance of the six membered ring and/or to the lower reactivity of the more electronically poor bromophenyl moiety with the electron withdrawing chromone. A final deprotection of the esters, carbonate and amide groups via a simple treatment with aqueous ammonia provided the three targeted conjugated fluorescent compounds **2** (**PCU**), **3** (**FCU)** and **4** (**TCU**), which were used for photophysical characterizations.
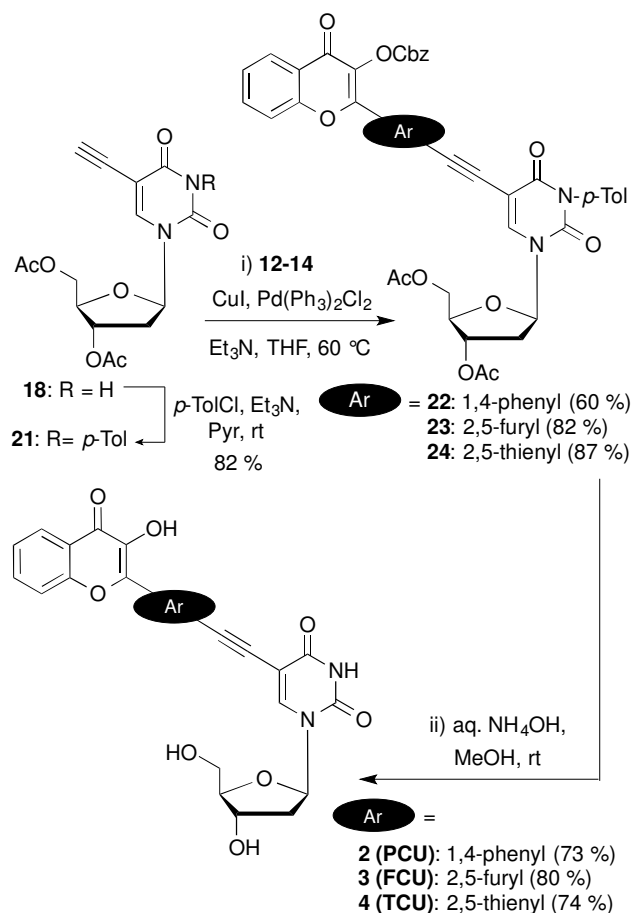
Scheme 4. Synthesis of dual emissive fluorescent nucleosides **2**, **3** and **4** bearing a 3HC scaffold.

Photophysical studies

Results

UV/Vis absorption and fluorescence properties of the three chromone-nucleoside analogues **PCU**, **FCU**, and **TCU** were investigated in methanol and compared with those of the

corresponding parent 2-phenyl, 2-furyl and 2-thienyl-3-hydroxychromones, **PC**, **FC**, and **TC** respectively (Table 1, Fig. S1).

These studies brought interesting results. In point of fact, the positions of the light absorption bands of the new derivatives were located in the same sequence as that of the parent 3HCs but demonstrated red-shifts by 25-36 nm, which could be explained by an electronic conjugation with uracil moiety (Fig. 3). Such conjugation should increase dramatically the light absorption cross-section leading to increase correspondingly the molar absorption coefficients. **PCU**, **FCU**, and **TCU** absorbed light about twice stronger, with molar absorption coefficients of about 27000, 37000 and 39000 M^{-1}.cm^{-1}. It must be noted that the shift of the absorption maxima to the red, and the increase of the molar absorption coefficients proceed in the following sequence: **PCU < FCU < TCU**. The more red-shifted absorption and emission of the thienyl and furyl derivatives as well as the increased absorption coefficients are in agreement with the higher electronic density of substituents in position 2 of the chromone ring, respectively. In our case, this may result in more efficient electronic coupling between the chromone and uracil electronic systems. The differences with the phenyl substituent can be accounted for the fact that the five-membered rings are more electron rich and allow to adopt a more planar conformation with respect to chromone for better coupling.[44] Thus, the novel conjugates display substantial shifts to the red of fluorescence spectra, for instance in methanol, the shifts of N* and T* bands are ca. 80 and 25 nm, respectively (Table 1, Fig. 3). The fluorescence quantum yields were also increased effectively, so that the brightness (defined commonly as the product of molar absorbance and quantum yield) could be increased roughly by one order of magnitude.
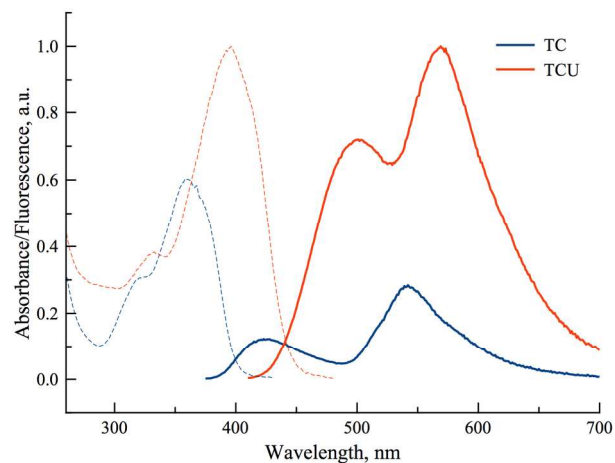
Fig. 3 Absorption and fluorescence emission spectra of **TC** (blue), and **TCU** (red) in methanol (excitation wavelength corresponds to the absorbance maximum of each compound). Absorptions and emissions are proportional to their absorption coefficients and quantum yields, respectively.

On the next step, the properties of **PCU**, **FCU** and **TCU** conjugates were investigated and compared in a set of solvents presenting a large range of polarities (Table 2). For these studies, five aprotic solvents (acetonitrile, acetone, chloroform, ethyl acetate, toluene) and four protic solvents (hexafluoropropan-2-ol (HFIP), H$_2$O, MeOH, EtOH) were selected.

Table 1. Spectroscopic comparison in methanol of parent 2-aryl-3-HCs (**PC**, **TC** and **TC**) and corresponding conjugates (**PCU**, **FCU** and **TCU**).

Dye	λ_{abs} [a]	$\Delta\lambda_{abs}$ [b]	ε [c]	$\varepsilon_\square/\varepsilon_\square$ [d]	λ_{N*} [e]	$\Delta\lambda_{N*}$ [f]	Φ [g]	$\Phi_\square/\Phi_\square$ [h]
PC[46,47]	343	-	13300	-	403	-	0.03	-
PCU	368	25	27000	2	500	97	0.30	10
FC[46,48]	356	-	16400	-	416	-	0.08	-
FCU	387	31	37000	2.2	497	81	0.24	3
TC[26]	359	-	23500	-	424	-	0.07	-
TCU	395	36	39000	1.7	501	77	0.32	4.5

Footnotes: a) Position of the absorption maximum in nm; b) difference of absorption maxima between the conjugate and the parent 3HC in nm; c) molar absorption coefficient in $M^{-1}.cm^{-1}$; d) ratio of molar absorption coefficients between the conjugate and the parent 3HC; e) position of the normal N* band maximum in nm; f) difference of emission maxima between the conjugate and the parent 3HC in nm; g) quantum yields determined using 3-hydroxyflavone in toluene (Φ=0.29),[47] 4'-(dimethylamino)-3-hydroxyflavone in ethanol (Φ=0.27)[49] or quinine sulfate in 0.1 м H_2SO_4 (Φ=0.54)[50] as standard references; h) ratio of quantum yields between the conjugate and the parent 3HC.

Table 2. Photophysical properties of **PCU**, **FCU** and **TCU** in different solvents.

Solvent	$E_T(30)$ [a]	λ_{N*} [c]			λ_{T*} [d]			I_{N*}/I_{T*} [e]			Φ [f]		
		PCU	**FCU**	**TCU**	**PCU**	**FCU**	**TCU**	**PCU**	**FCU**	**TCU**	**PCU**	**FCU**	**TCU**
HFIP	65.3	472	493	500	–	–	–	–	–	–	0.28	0.33	0.40
H_2O	63.1	480	499	502	525	–	–	0.74	–	–	0.05	0.13	0.06
MeOH	55.4	500	497	504	547	551	568	0.64	1.03	0.79	0.30	0.24	0.32
EtOH	51.9	489	488	501	552	566	575	0.33	0.56	0.44	0.26	0.22	0.23
CH_3CN	45.6	445	466	474	549	569	580	0.03	0.20	0.28	0.33	0.17	0.19
Acetone	42.2	455	462	469	553	574	583	0.04	0.17	0.29	0.22	0.16	0.25
$CHCl_3$	39.1	426	454	459	543	564	572	0.02	0.15	0.17	0.40	0.23	0.19
EtOAc	38.1	424	444	457	551	573	582	0.03	0.10	0.20	0.34	0.22	0.20

Footnotes: a) Reichardt's empirical solvent polarity index;[45] b) position of the absorption maximum; c) position of the N* band maximum; d) position of the T* band maximum; e) ratio of the two intensity maxima; f) quantum yields determined using 3-hydroxyflavone in toluene (Φ=0.29),[47] 4'-(dimethylamino)-3-hydroxyflavone in ethanol (Φ=0.27)[49] or quinine sulfate in 0.1 м H_2SO_4 (Φ=0.54) as references.[50]

The Reichardt's polarity parameter $E_T(30)$ that accounts for the dielectric constant of the solvent and its H-bond donor ability was employed to scale the polarity of the solvents.[45] We observed that the positions of the absorption maxima were similar to the ones observed in MeOH (Table 1). Therefore, they correlate poorly with the solvent polarity displaying almost no solvatochromism, which is the evidence of an absence of charge-transfer character in their ground states.

PCU presented a dual emission in protic solvents, except HFIP. The short and long wavelength bands can be attributed to the N* and T* states. In contrast, it presented almost exclusively the T* band in all aprotic solvents (Fig. 4). In all solvents, except water and HFIP, **FCU** and **TCU** exhibited the two emission bands (Fig. 4). The positive solvatochromism of the N* band is typical for ESIPT probes with ICT character.[26,27] The ICT character can be further estimated using the polarity scale of Lippert (Table S1 and Fig S5, S6).[1a,51] Plotting the Stokes shifts as a function of the orientation polarizability of the aprotic solvents demonstrated linear fits with positive slopes for both compounds. These results are compatible with an increase of the dipole moments of the normal excited states of **FCU** and **TCU** after Franck-Condon light absorption. The position of their T* band maxima appeared blue-shifted in protic solvents, as compared to aprotic ones. The blue shift can be explained by the formation of an H-bond between the 3-phenoxide oxygen in the T* state and the donor proton in the solvent.[52,53]
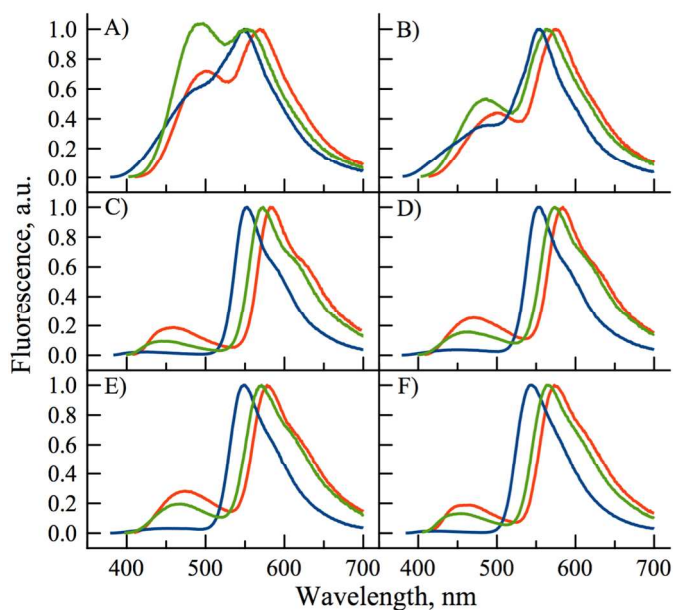

Fig. 4 Normalized fluorescence spectra of **PCU** (blue), **FCU** (green) and **TCU** (red) in: A) methanol, B) ethanol, C) ethyl acetate, D) acetone, E) acetonitrile and F) chloroform. All the spectra are normalized by T* band (excitation wavelength corresponds to the absorbance maximum of each compound).

All the three compounds displayed an increase in the I_{N*}/I_{T*} ratio by rising solvent polarity and H-bond donor strength (Table 2, Fig. 4). However, **FCU** and **TCU** were much more sensitive than **PCU**. As a general trend, the I_{N*}/I_{T*} ratio of **FCU** and **TCU** gradually increased when going from the most apolar aprotic solvent to the most polar protic solvent (Table 2, Fig. S2, S3, S4). It is worth indicating that the effect of H-bonding dominates the polarity effect, as that could be seen from the 3- to 5-fold larger value of the I_{N*}/I_{T*} ratio in methanol as compared to acetonitrile, though both solvents exhibit similar dielectric constants. Analysing the plots of Lippert further supports this interpretation. In protic solvents, the Stokes shifts were deviated to the red from the linear function indicating specific interactions between the protic solvent and the 3HC (Fig S5, S6). The deviation from the linear fit in protic solvents is known for donor-acceptor solvatochromic dyes.[1a] The sensitivity of **FCU** and **TCU** is typical of the 3HCs exhibiting significant ICT characters, such as 2-(4-methoxy)phenyl-3HCs[54] and 2-(4-dialkylamino)phenyl-3HCs.[16,55]

For **PCU**, the contribution of the N* form was marginal in aprotic solvents hinting that **PCU** was mainly sensitive to protic solvents that donate protons for intermolecular H-bonding with the carbonyl at position 4 of 3HC. **PCU** behaves like the parent 3-hydroxyflavone,[56] but with a lower resolution of its dual emission.

The quantum yields of the three dyes were significantly enhanced for all the compounds in comparison with their parent analogs (Table 1). As a function of solvent, they range from 5 to 40 %. Quantum yields were in the range of 22 to 40 % in all the tested alcohols and 5 to 13 % in water. However, the quantum yields in HFIP, which is more polar and acidic than water,[57] are about 3 to 7 times larger than in water. Therefore, the reduced quantum yields in water are unlikely due to the H-bond strength between the donating water molecule and the accepting oxygen carbonyl of 3HC (Scheme 5). Reduction of quantum yields in water for solvatochromic dyes exhibiting strong ICT character is common. It was proposed that several water molecules act cooperatively to capture electrons from the excited-state species resulting in quenching of fluorescence.[58] Though being reduced in comparison with other solvents, the quantum yields of the three compounds are still significant in water making them attractive for sensing hydrated biological media. This is a major difference as compared to many other solvatochromic dyes, which are severely quenched in water.[59]

To get further insight on the sensitivity of the photophysical properties of **PTU**, **FCU**, and **TCU** on hydration, emission spectra were recorded in mixtures of acetonitrile and water (Fig. 5). The same set of experiments was also performed in dioxane/water mixtures in order to determine the contribution from the organic solvent to the spectral changes (Fig. S7). A dual emission was evident in all the tested compositions for **PCU** and up to 70% and 90% water for **FCU** and **TCU**, respectively. In all cases, hydration increased the N*/T* ratio and shifted the T* band position to the blue, as it was observed in neat protic solvents (Table 2). The ratios of the intensities correlated linearly with the water concentration demonstrating the absence of high-affinity specific hydration (Fig. 6).

These ratios increased gradually from 0.03, 0.2 and 0.27 in neat acetonitrile to 0.77, 1.4 and 1.15 in 100, 70 and 90% water for **PCU**, **FCU** and **TCU**, respectively (Fig. 5 & 6 and Table S2). To conclude, the sensitivity of the N*/T* ratio to water followed the order: **FCU > TCU > PCU** (slope comparison in Fig. 6). Comparable variations of the ratios of the intensities

were found when dioxane was used as an organic solvent (Table S2), confirming that the N*/T* ratio is mainly controlled by water (Fig. S8 & S9).
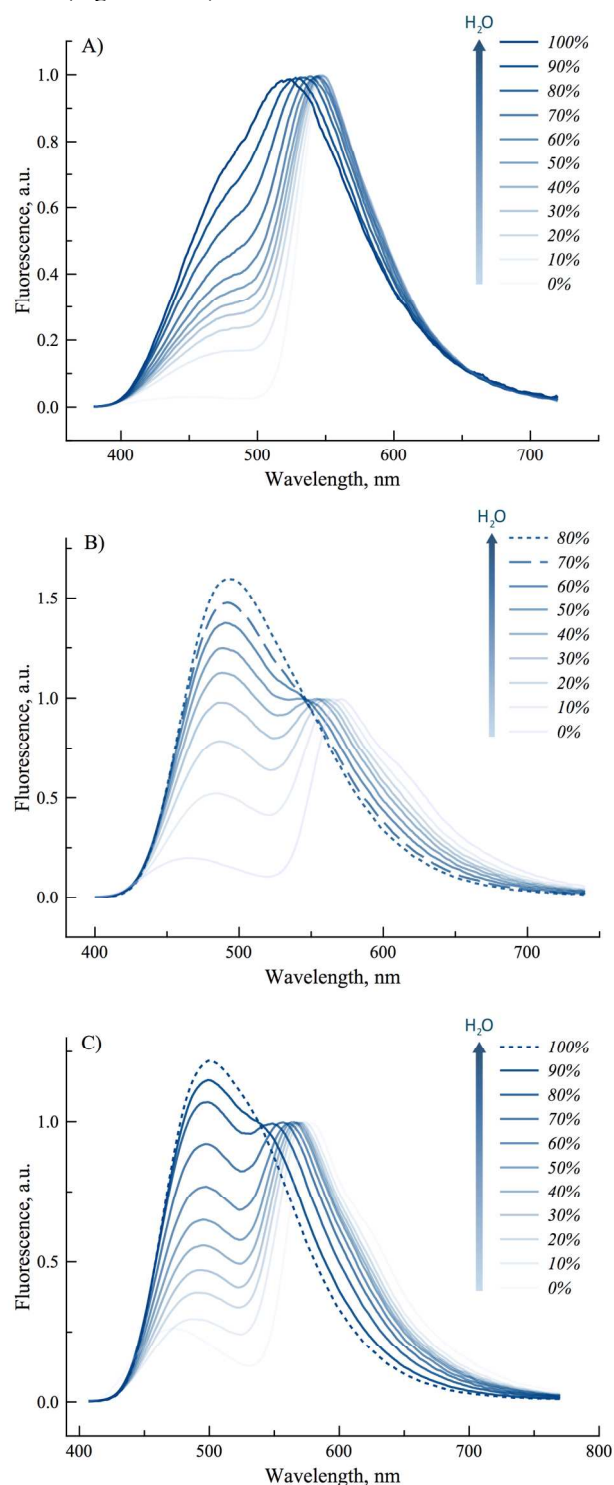

Fig. 5 Sensitivity of the emission spectra to hydration for A) **PCU**, B) **FCU** and C) **TCU**. Data were recorded in gradual mixtures of acetonitrile and water. Emission spectra were normalized by T* band.

The remarkable sensitivity of 3HCs to hydration correlates

with previous studies carried out with derivatives of 3HCs for which a model was proposed.[52,56,60] This latter involves specific H-bonding interactions of water with the electron rich oxygens of 4-carbonyl and phenoxide of 3HC (Scheme 5). As a result, the N* form exists in equilibrium with a protonated N*-H form, in which intermolecular H-bonding of the 4-carbonyl with water inhibits the ESIPT process.[61] Thus, growing water concentration favors the N*-H form, and consequently, increases the N*/T* ratio. Moreover, hydration also favors H-bonding of the tautomer T* form, which results in its blue-shifted emission maximum.[52]
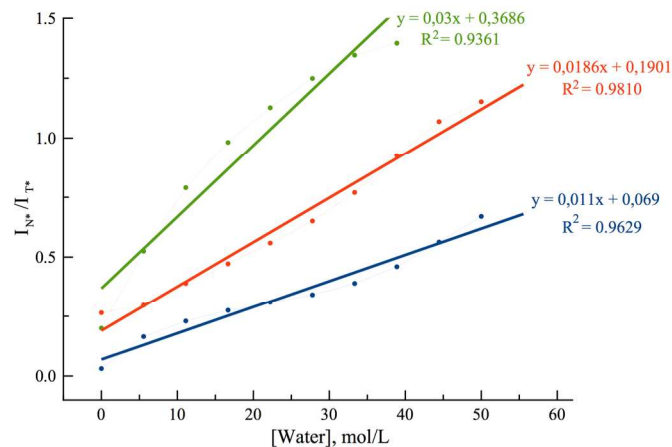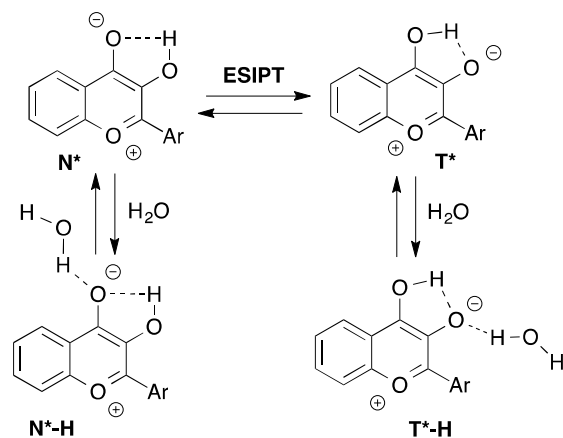

Fig. 6 Dependence of the ratiometric response (I_{N*}/I_{T*}) of **PCU** (blue), **FCU** (green), and **TCU** (orange) on the water concentration in acetonitrile. The ratios were extracted from the spectra in Fig. 4.

Scheme 5. Proposed mechanism for water sensing by 3HCs adapted from previous works.[52,60]

Conclusions

We developed three dual emissive nucleoside derivatives based on the assembly of a 2-aryl-3HC dye and a 2'-deoxyuridine fragment, connected through a rigid and electron-conducting ethynyl linker. The three conjugates **PCU**, **FCU** and **TCU** differ from each other by the C-2 aromatic ring (phenyl, furyl and thienyl) of the 3HC. Their photophysical properties were investigated in a wide range of solvents. The conjugation of furyl- and thienyl-chromones with the uracil moiety resulted in

the most dramatic improvement of their spectroscopic properties.

In the present work, our results show the amplification of the brightness by about one order of magnitude due to two-fold increase of the absorbance and 4 times the enhancement of the fluorescence quantum yield, whilst retaining the most remarkable property of 3HC dyes to respond to polarity and hydration of their environment by changes in their dual emission.[13,16] The enhancement of the quantum yield is quite unexpected, since an increase of the conjugation in the π-electronic systems generally results in an increase of the fluorescence quenching.[62] Additionally, these derivatives keep significant quantum yields in water, which is not common among polarity-sensitive dyes[59,63] but was observed for a series of 3HC derivatives.[46,54] This makes our new compounds highly prospective for applications as fluorescent reporters in biological media. Since water plays a key role in controlling nucleic acid structure and function through H-bonding and electrostatic interactions, the exquisite ratiometric sensitivity of **FCU** and **TCU** to hydration makes them highly relevant for subsequent major groove labeling. Moreover, the ethynyl linker is thought to locate the dye in a precise position with respect to the DNA major axis, which is of key importance for further data interpretation of biomolecular interaction studies. Incorporation of these prospective candidates into oligonucleotides, as environmentally sensitive fluorescent (ESF) probes, is currently the focus of our ongoing researches and will be reported in due time.

Notes and references

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† An one-pot two-step procedure, consisting in Pd-catalyzed coupling followed the removal of the silyl group, was also attempted however a moderate yield of 64 % was obtained.

†† Concerning the furano[2,3-*d*]pyrimidin-2-one nucleoside **20**, NMR and MS analysis gave the spectroscopic characteristics consistent with a 5-*endo* cyclized derivative. Indeed, the disappearance in ¹H-NMR of the imide N*H* signal at 9.35 ppm was observed in favor of a new singlet at 6.92 ppm corresponding to the proton of the fused furan ring. Moreover in ¹³C-NMR, the lost of the two typical signals of the ethynyl carbons at

83 and 87 ppm in favor of two new aromatic peaks respectively at 99 and 146 ppm bring the undeniable evidence of a furanopyrimidine scaffold. It is noteworthy that the deshielding (>10 ppm) of C-4 and C-5 of uracil fragment to respectively 107 ppm and 171 constitutes another proof of the proposed bicyclic aromatic structure.

Electronic Supplementary Information (ESI) available: the experimental protocols for the synthesis and characterization, the ¹H and ¹³C NMR spectra of the compounds **2-4** and **9-24,** and the HPLC of the final products **2-4** can be consulted in the Supporting Information. See DOI: 10.1039/b000000x/

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