Highly Solvatochromic 7-Aryl-3-hydroxychromones

ARTICLE *in* JOURNAL OF PHYSICAL CHEMISTRY LETTERS · MARCH 2012

Impact Factor: 7.46 · DOI: 10.1021/jz3002019

THE JOURNAL OF PHYSICAL CHEMISTRY Letters

Letter

pubs.acs.org/JPCL

Highly Solvatochromic 7-Aryl-3-hydroxychromones

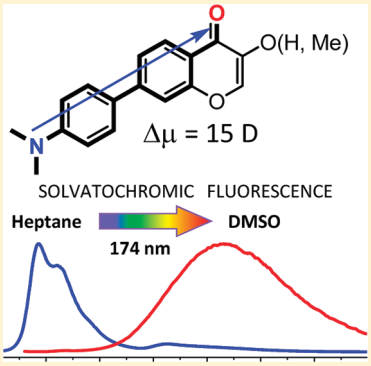
Luciana Giordano,*[,†] Volodymyr V. Shvadchak,[†] Jonathan A. Fauerbach,[‡] Elizabeth A. Jares-Erijman,[‡,#] and Thomas M. Jovin[†]

[†]Laboratory for Cellular Dynamics, Max Planck Institute for Biophysical Chemistry, Am Faßberg 11, Göttingen, Germany

[‡]Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Supporting Information

ABSTRACT: Introduction of the dialkylaminophenyl group in position 7 of 3-hydroxychromone changes the orientation of the excited-state dipole moment and leads to superior solvatochromic properties (>170 nm emission shift in aprotic media). The excited-state intramolecular proton-transfer (ESIPT) reaction of 7-aryl-3-hydroxychromones is almost completely inhibited in most solvents. Methylation of the 3-OH abolishes ESIPT completely and also leads to improved photostability. The probes exhibit a ∼100-fold increase in fluorescence intensity and large Stokes shifts upon binding to membranes, reflecting differences in membrane phase and charge by a >40 nm spread in the emission band position.



SECTION: Spectroscopy, Photochemistry, and Excited States

Fluorescent probes are indispensable in the study of molecular states and interactions in solution as well as in the biological context.[1−3] Of particular note is the class of probes exhibiting solvatochromism in response to changes in the local molecular microenvironment accompanying the interactions[4] and conformational changes[5] of covalently or noncovalently labeled proteins and membranes. Solvatochromism is defined as the alteration of the absorption and/or emission properties of a chromo(fluoro)phore due to the influence of its molecular environment and supporting solvent.[6,7] In the case of fluorescence, the spectral shifts and intensity changes (enhancement, quenching) reflect differential perturbations of the ground and excited states, such as in solvent relaxation, molecular and conformational and positional orientation, and noncovalent interactions (H-bonding, solvent relaxation). The observed solvatochromism is denoted as positive (more common) or negative depending on whether the emission undergoes a bathochromic (red) or hypsochromic (blue) shift, respectively, with an increase in solvent polarity. Most of the environment-sensitive dyes exhibit strong changes in dipole moment upon electronic excitation due to intramolecular charge transfer (ICT) from an electron-donor to an electron-acceptor group. These distinctions have been rationalized in terms of the relative electrophilicities of the donor and acceptor moieties.[8] Nonetheless, the general model of solvatochromism does not account for specific interactions such as the very predominant phenomenon of H-bonding, for which the 3-hydroxychromones (3HC) are particularly sensitive.[9] For general discussions of the theory and quantitative formalism(s) of solvatochromism, see refs 6 and 9−11.

One of the best-known micropolarity-sensitive fluorophores, introduced by Weber and Farris in 1979,[12] is 2-propionyl-6-dimethylaminonaphthalene (Prodan). The chromophore has a push−pull charge-transfer (CT) character, with electron-donating dimethylamino and electron-withdrawing propionyl groups located at positions 2 and 6 of naphthalene. Prodan exhibits a large increase (4−9 D) in the molecular dipole moment upon excitation,[13,14] and recent molecular computations support experimental evidence for either planar[7,15] or mixed planar and twisted (in polar media and lipid bilayers[16]) configurations as the emitting species. Thus, both conformational and electronic states contribute to the net solvatochromism of such probes, which include a number of new compounds: extended analogues of Prodan based on fluorene[14,17] and anthracene,[18] substituted naphthalimides,[19−21] and aminocyanonapthalene.[22] In the case of the 3-hydroxychromones (3HC), the 3-OH group forms intermolecular bonds with H-bond proton acceptors and thus reports the basicity of the microenvironment, while the 4-carbonyl forms H-bonds with proton-donor groups and reports on environmental acidity. In addition, 3HC derivatives undergo excited-state intramolecular proton transfer (ESIPT), resulting in two excited-state forms with distinct, well-separated emission bands, a normal (N*) and the ESIPT product tautomer (T*).

Received: February 17, 2012
Accepted: March 20, 2012
Published: March 20, 2012

Scheme 1. Molecular Structures of the New Fluorescent Probes 7AHC and 7AMC Compared with Prodan

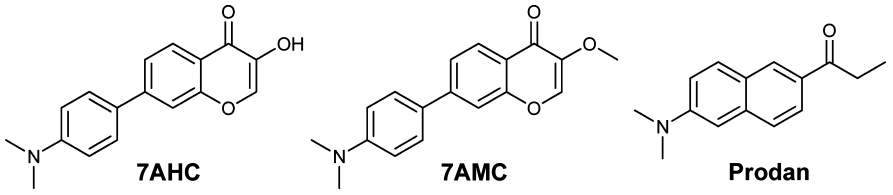


Table 1. Spectroscopic Properties of 7AHC and 7AMC in Comparison with Prodan in Selected Solvents[a]

		$\lambda_{max,\ abs}$, nm			$\lambda_{max,\ fluo}$, nm			QY, %		
solvent	$E_T(30)$[b]	7AHC	7AMC	PRO	7AHC	7AMC	PRO	7AHC	7AMC	PRO
heptane	31.0	357	348	343	393	388	391	26	2.2	1.9
toluene	33.9	367	361	349	442	432	417	57	46	41
1,4-dioxane	36.0	362	360	348	457	451	426	56	58	61
THF	37.4	365	362	350	490	470	429	57	62	58
DCM	40.7	373	368	354	503	488	429	64	59	70
DMSO	45.1	380	375	359	567	545	461	20	20	75
acetonitrile	45.6	366	363	351	559	530	454	16	16	53
1-octanol	48.1	362	373	360	532[c]	540	473	9.8	12.4	38.5

[a]The extended table with all of the studied solvents is presented in the SI. [b]$E_T(30)$ is the polarity index (see refs 6 and 34). QY is the emission quantum yield. [c]Broad emission band.

It has been previously shown that introduction of a donor substituent (methoxy,[23,24] dimethylamino[25]) in position 7 of the 2-aryl-3HC ring increases the stability of the tautomeric state and leads to a nearly 10 nm blue shift in absorption, whereas electron acceptors (methoxycarbonylvinyl[26] or isothiocyanate[27]) have an opposite effect. Interestingly, none of the substituents increase the solvatochromism of the N* band. In view of the very low fluorescence quantum yield and the near-UV nm absorption of unsubstituted 3HC,[28] all proposed 3HC-based fluorophores have featured substituents at position 2.

In this Letter, we introduce 7-aryl-3-hydroxychromone (**7AHC**), a new fluorophore inspired by the pull−push CT characteristic of Prodan and with an extended aromatic system at position 7 of 3HC. We anticipated a change in the orientation of the dipole moment compared to that of 2-aryl-3HC, an increase in fluorescence intensity over that of 3HC lacking a substituent at position 2, as well as an influence on the dynamics of the ESIPT reaction. The 3-OH was methylated in **7AMC** so as to completely abrogate intramolecular proton transfer and thus evaluate its influence on the observed solvatochromism (see Scheme 1).

Fluorescent compounds **7AHC** and **7AMC** were synthesized starting from 3-bromophenol in four or five steps, respectively (details are given in the Supporting Information, SI). Absorption and fluorescence spectra were recorded in different organic solvents and compared to those of the reference compound Prodan. A weak positive solvatochromism was observed for the absorption of **7AHC** and **7AMC**, suggesting that the electronic excitation increases the dipole moment of the molecules and that the orientations of the ground- and excited-state dipoles are similar. The absorption coefficient at the long-wavelength absorption maximum is 16 000 M^{-1} cm^{-1} in both aprotic polar and nonpolar solvents, a value close to that of Prodan (18 400 M^{-1} cm^{-1}).[12]

In contrast to unsubstituted 3HC, **7AHC** and **7AMC** display a high fluorescence intensity in most organic aprotic solvents. The fluorescence quantum yield increases from ∼0.2 in apolar alkanes to 0.5−0.6 in solvents of medium polarity and diminishes gradually in polar solvents (Table 1), a common property of dyes exhibiting large charge separation in the excited state.[19,29,30] Unlike Prodan, **7AHC** and **7AMC** have lower quantum yields in protic solvents (from ∼0.04 in BuOH to ∼0.001 in MeOH). One possible mechanism for the quenching is proton transfer from the solvent to the carbonyl group of the dye.[31,32] In view of the observation, addition of 1% (v/v) D_2O to a solution of **7AHC** in an aprotic solvent (THF) quenched the fluorescence less than H_2O (33 compared to 44%, Figure S1, SI).

The emission spectra are characterized by high positive solvatochromism, with maxima significantly red-shifted with respect to Prodan (Figure 1). Remarkably, the emission of the tautomeric (T*) form of **7AHC** is appreciable only in apolar heptane and does not exceed 10% of the normal (N*) form

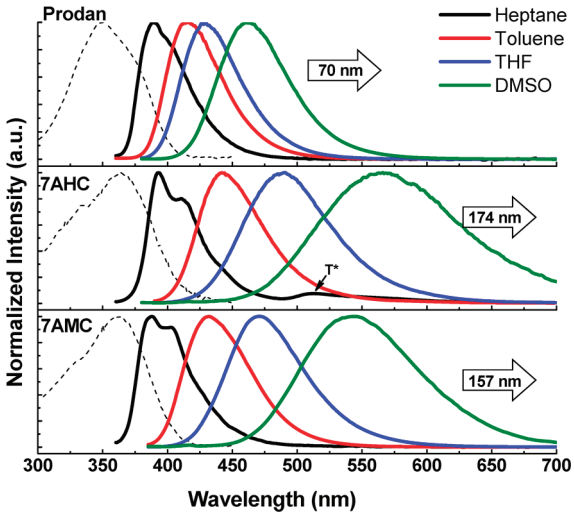


Figure 1. Absorption spectra in THF (dashed line) and fluorescence spectra (solid lines) of compounds **7AHC**, **7AMC**, and Prodan excited at 370 nm. The values in arrows are the magnitudes of the solvatochromic shift in passing from heptane to DMSO.

dx.doi.org/10.1021/jz3002019 | J. Phys. Chem. Lett. 2012, 3, 1011−1016

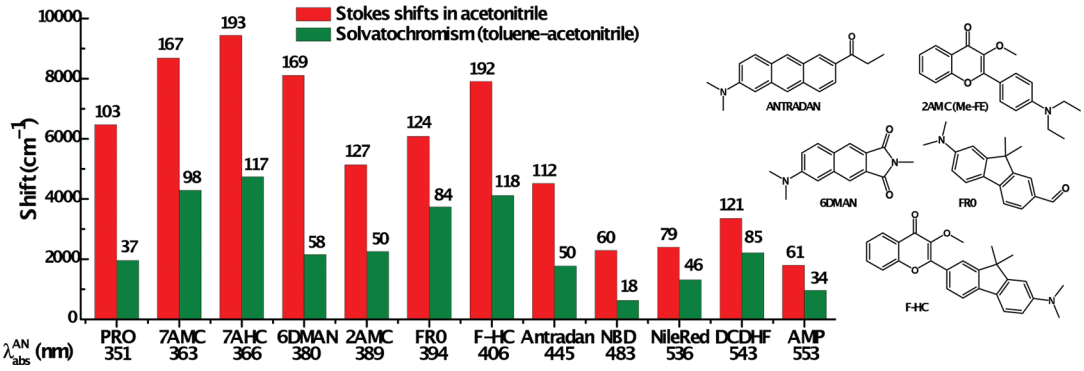


Figure 2. Comparison of Stokes shifts ($\Delta\tilde{\nu} = \tilde{\nu}_{abs} - \tilde{\nu}_{fluo}$) and the solvatochromic magnitudes ($\tilde{\nu}^{Tol}_{fluo} - \tilde{\nu}^{AN}_{fluo}$) of various solvatochromic dyes, ordered by the absorption maximum in acetonitrile (λ^{AN}_{abs}). The numbers over the bars correspond to Stokes shifts and solvatochromism in nm. Data for 6DMAN,[19] FR0,[14] 2AMC,[31] F-HC,[31] Antradan,[18] NDB,[35] Nile Red,[36] DCDHF,[34] and AMP[5] are from the cited original papers.

intensity. We conclude that introduction of a donor aryl group in position 7 increases the stability of the N* form, in contrast to the behavior of 3HCs bearing an aryl substituent at position 2 in which donors at position 7 decrease the stability of the N* form.[24] In addition, the emission maxima of the new dyes vary strongly with solvent polarity, for example, shifting to the red by 174 (7800 cm^{-1}) and 157 nm (7400 cm^{-1}) for **7AHC** and **7AMC**, respectively, upon changing from apolar heptane to polar DMSO. The full width at half-maximum (in cm^{-1}) of the emission bands also increases with solvent polarity due to the shift in the positions of the maxima on the nanosecond time scale (see time-resolved emission spectra in Figures S6–S9, SI) probably caused by a CT reaction (ICT)[33] and solvent relaxation. In apolar heptane and polar acetonitrile, for example, the corresponding bandwidth values are 2600 and 4000 cm^{-1} for **7AHC** and 2800 and 3600 cm^{-1} for **7AMC**. Prodan shows only a small increase (2700 and 3100 cm^{-1}) in the same solvents.

Compounds **7AHC** and **7AMC** exhibit greater solvatochromism than most other probes (Figure 2, green bars). The magnitude of solvatochromism is double that of Prodan,[12] 6DMAN,[19] antradan[18] or DCDHF[34] and comparable to that of fluorene derivatives F-HC[31] and FR0.[14] The Stokes shifts in acetonitrile are also greater (Figure 2, red bars). Fluorophores with a relatively small charge separation in the ground state are more solvatochromic due to the greater relative changes in the molecular dipole moment upon excitation.

Solvent-dependent spectral shifts are most often interpreted in terms of a single parameter, one of the most popular being the polarity index $E_T(30)$,[6,37] a measure of the ionizing power ("polarity") of a solvent. Polar protic solvents were not used in our study because they substantially quench the fluorescence of **7AHC** and **7AMC**. A linear correlation of the emission band positions of **7AHC**, **7AMC**, and Prodan with $E_T(30)$ is observed (Figure 3). Remarkably, the new fluorescent compounds exhibit a much steeper slope than Prodan, providing a stronger solvatochromic response to changes in polarity. The dyes bear both electron-donor and -acceptor groups, such that in the excited state, CT occurs from the dimethylamino to the carbonyl group through the conjugation core, strongly increasing the dipole moment.

Another relationship commonly used to specify solvatochromism behavior is the Lippert–Mataga equation (see SI), which describes the dependence of Stokes shifts (expressed in wavenumbers) on the orientation polarizability, Δf (Figure S2, SI). This treatment allows an estimation of the difference

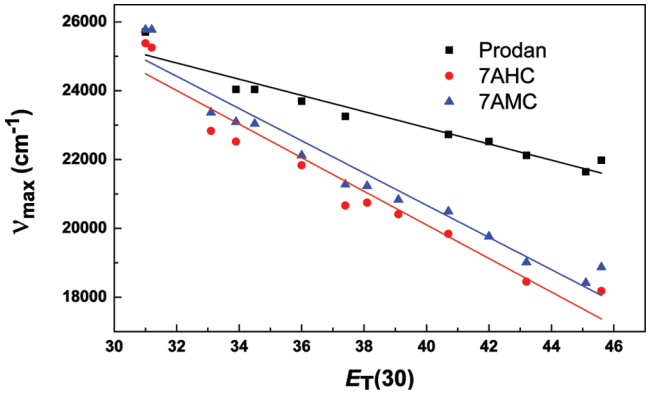


Figure 3. Positions of maximal emission as a function of the solvent polarity index $E_T(30)$ of aprotic solvents for Prodan, **7AHC**, and **7AMC**.

between the ground- and excited-state dipole moments ($\Delta\mu$) of the probes, 15.9 and 14.4 D for **7AHC** and **7AMC**, respectively. These values are double those of Prodan (6.5 D) and the isomer 2-diethylaminophenyl-3-hydroxychromone (FE, 7.6 D), measured in this and in previous studies.[13,14]

The two approaches based on $E_T(30)$ and Δf attempt to link the spectral properties of dyes to single parameters. Recently, multiparameter correlations have been found to be preferable in that they consolidate the different types of contributions to solvent effects from/in localized donor–acceptor interactions, acid–base interactions, and the action of the solvent as a dielectric continuum. The general four-parameter Catalán[11,38] solvent scale is particularly useful because it divides the unspecific solvent effects into two parameters, solvent polarizability (SP) and dipolarity (SdP). The other two terms in this approach reflect specific interaction effects, a solvent acidity term (SA) and a basicity term (SB). The Catalán formalism is represented by eq 1, a linear regression of a particular spectral measure (y) on the tabulated solvent parameters SP, SdP, SA, and SB. The coefficients a_{SP}, b_{SdP}, c_{SA}, and d_{SB} reflect the relative influence of each parameter.

$$y = y_0 + a_{SP}SP + b_{SdP}SdP + c_{SA}SA + d_{SB}SB \quad (1)$$

We analyzed the absorption maxima ($\tilde{\nu}_{abs} = 1/\lambda_{abs.\,max}$), the emission maxima ($\tilde{\nu}_{fluo} = 1/\lambda_{fluo,max}$), and the Stokes shifts ($\Delta\tilde{\nu} = \tilde{\nu}_{abs} - \tilde{\nu}_{fluo}$) (all in cm^{-1}) as linear regressions on three of the Catalán parameters catalogued for the various solvents.[38] Because only aprotic solvents were used, the SA term was

dx.doi.org/10.1021/jz3002019 | J. Phys. Chem. Lett. 2012, 3, 1011–1016

Table 2. Dependence of the Spectral Properties of 7AHC, 7MHC, and Prodan on Solvent Parameters[a]

probe	y	$10^{-3}y_0$	$10^{-3}a_{SP}$	$10^{-3}b_{SdP}$	R
7AHC	$\tilde{\nu}_{abs}$	31.3 ± 0.7	−(5.2 ± 1.0)	−(0.6 ± 0.2)	0.802
	$\tilde{\nu}_{fluo}$	25.0 ± 0.3		−(7.1 ± 0.4)	0.965
	$\Delta\tilde{\nu}$	2.9 ± 0.3		6.4 ± 0.5	0.954
7AMC	$\tilde{\nu}_{abs}$	31.7 ± 0.3	−(4.7 ± 0.4)	−(1.2 ± 0.1)	0.977
	$\tilde{\nu}_{fluo}$	25.0 ± 0.3		−(6.5 ± 0.4)	0.957
	$\Delta\tilde{\nu}$	3.3 ± 0.2		5.1 ± 0.3	0.963
Prodan	$\tilde{\nu}_{abs}$	31.3 ± 0.4	−(3.3 ± 0.4)	−(0.75 ± 0.1)	0.927
	$\tilde{\nu}_{fluo}$	25.2 ± 0.2		−(3.2 ± 0.3)	0.884
	$\Delta\tilde{\nu}$	3.9 ± 0.2		2.3 ± 0.3	0.839

[a]Regressions according to eq 1 of $\tilde{\nu}_{abs}$, $\tilde{\nu}_{fluo}$, and $\Delta\tilde{\nu}$ of Prodan, **7AHC**, and **7AMC** as a function of the Catalán solvent scale. Coefficients (y_0, a_{SP}, b_{SdP}, d_{SB}) with their standard errors (all in cm^{-1}) and correlation coefficients (R). The calculations were performed with *Mathematica* software.

Table 3. Lifetimes and Photostability of 7AHC and 7AMC in Comparison to Prodan[a]

	7AHC			**7AMC**			Prodan		
solvent	$\langle\tau_f\rangle$ (ns)	k_{bl} (s^{-1})	Q_{bl}	$\langle\tau_f\rangle$ (ns)	k_{bl} (s^{-1})	Q_{bl}	$\langle\tau_f\rangle$ (ns)	k_{bl} (s^{-1})	Q_{bl}
toluene	1.46 ± 0.05	4.9×10^6	7.1×10^{-3}	0.91 ± 0.04	4.5×10^6	4.1×10^{-3}	1.85 ± 0.07	1.5×10^6	2.7×10^{-3}
THF	2.31 ± 0.02	4.3×10^5	1.0×10^{-3}	2.02 ± 0.02	3.8×10^4	7.6×10^{-5}	2.66 ± 0.03	1.9×10^4	5.2×10^{-5}
DMSO	1.87 ± 0.07	2.8×10^6	5.3×10^{-3}	2.36 ± 0.04	2.9×10^5	3.3×10^{-4}	3.12 ± 0.03	3.2×10^5	1.0×10^{-3}

[a]$\langle\tau_f\rangle$: mean fluorescence lifetime measured at the maximum of the emission peak. k_{bl}: rate constant of excited-state degradation. Q_{bl}: photobleaching quantum yield ($\langle\tau_f\rangle k_{bl}$).

omitted. The results of the regression analyses are given in Table 2 and Table S2 (SI).

The three compounds display similar solvent effects in terms of the Catalán parameters. In general, the analysis of band positions according to eq 1 yields comparatively small estimates for d_{SB} with a relatively large standard error. Hence, solvent basicity can be disregarded as a critical factor responsible for the spectral parameters $\tilde{\nu}_{abs}$, $\tilde{\nu}_{fluo}$, and $\Delta\tilde{\nu}$ ($\tilde{\nu}_{abs} - \tilde{\nu}_{fluo}$).

A satisfactory fit was obtained for the solvent dependence of the absorption maxima ($\tilde{\nu}_{abs}$). Despite the large (negative) value of a_{SP} compared to that of b_{SdP}, both parameters are essential because the linear single-parameter regressions of $\tilde{\nu}_{abs}$ versus SP or SdP yield low correlation coefficients (e.g., r = 0.54 and 0.27, respectively, for **7AHC**).

The fits of $\tilde{\nu}_{fluo}$ as a function of SP and SdP are also satisfactory. However, in contrast to $\tilde{\nu}_{abs}$, the single-parameter linear regressions of $\tilde{\nu}_{fluo}$ on SdP or SP show that the solvent-dependent shifts of the emission are influenced by solvent dipolarizability (r = 0.965 for **7AHC**) but not by solvent polarizability (r = 0.07 for **7AHC**). That is, solvent polarizabity can be disregarded as a critical factor in the emission shift. The b_{SdP} coefficients are much higher than those calculated for the absorption in the case of all three compounds. We conclude that the electronic structures of the Franck−Condon and relaxed excited state differ significantly, with the relaxed excited state being more polar and having a larger excited-state dipole moment compared to the ground state. The large dipole moment of the excited state is also manifested by time-dependent fluorescence spectra (TRES) manifesting up to a 20 nm red shift of the emission maximum (Figures S6−S9, SI).

The correlations according to eq 1 of the Stokes shifts of Prodan, **7AHC**, and **7AMC** with SP and SdP as independent variables are also satisfactory. However, the a_{SP} coefficients are small and have large standard errors, indicating that solvent polarizability (as well as solvent basicity) are not relevant. Thus, the fit with SdP as the only independent variable suffices.

A comparison of the b_{SdP} coefficients for $\tilde{\nu}_{fluo}$ and the Stokes shift of the three compounds reveals 2-fold higher values for **7AHC** and **7AMC** than that for Prodan, accounting for the greater solvatochromism.

Time-resolved fluorescence measurements of **7AHC**, **7AMC**, and Prodan were performed in aprotic solvents. In the case of **7AHC** and **7AMC**, the mean fluorescence lifetime increased, and the radiative decay constant (k_r) decreased with solvent polarity (Table 3 and Table S3, SI), contributing to the lower quantum yields of **7AHC** and **7AMC** in polar protic solvents.

In probes exhibiting ESIPT, the rate of proton transfer and, therefore, the T* band intensity increase in an apolar environment. Most of the described 3HC-based fluorophores contain an electron-donating aryl group at position 2 because unsubstituted 3HC has a low dipole moment and shows almost no emission at room temperature.[28] The modifications increase the dipole moment and stabilize the N* state. In the case of 7-aryl-3-hydroxychromone lacking a substituent at position 2, the dipole moment is completely reoriented. As a consequence, the N* form is stabilized, and the formation of the tautomeric form is reduced, such that its emission can be observed only in apolar heptane and does not exceed 10% of the intensity of the N* form. In contrast, the T* band emission of the isomeric 2-aryl-3-hydroxychromone in heptane is 100-fold higher than that of the N* form. Time-resolved emission spectra show a ∼0.3 ns rising component for the T* band, pointing to the formation of the T* form via an excited-state reaction (proton transfer) with a rate of $\lesssim 3 \times 10^9$ s^{-1}, a value at least 20-fold lower than that for the isomeric 2-aryl derivative FE[39] (Table S4, SI).

The photostabilities of the new dyes were characterized by recording the emission spectra after intervals of irradiation with a mercury lamp (365 nm). Photobleaching rate constants (k_{bl}) and the corresponding photobleaching quantum yields (Q_{bl}) were calculated after adjustment for irradiance and absorption (Table 3). Representative time courses are given in Figures S10−S12 (SI). The relative photobleaching rates varied according to the solvent, toluene (**7AHC** > **7AMC** > Prodan), THF (**7AHC** ≫ **7AMC** > Prodan), and DMSO (**7AHC** > **7AMC** = Prodan). Methylation of the 3-OH group (**7AHC** → **7HMC**) confers greater photostability on the probe.

dx.doi.org/10.1021/jz3002019 | J. Phys. Chem. Lett. 2012, 3, 1011−1016

The pronounced solvatochromism of **7AHC** leads to consideration of its use for probing microenvironmental polarity in biological systems. Due to its red-shifted emission and the greater separation between the absorption and emission spectra compared to that of Prodan, **7AHC** should be well-suited for cellular imaging by photon counting microscopy and spectroscopy. We tested its applicability in the discrimination of phase and charge of synthetic lipid membranes using model vesicle systems (Figure 4 and Table S5, SI). The binding of **7AHC** to membranes leads to a >100-fold increase in the fluorescence intensity, thus minimizing the influence of the free probe on the spectral shape. The emission band position varies more than 40 nm depending on the phase and charge of the lipid bilayer. The spectra corresponding to the most apolar environment (blue-shifted emission) are observed in rigid lipid bilayers ($L_{\beta'}$ phase), in accordance with the low hydration and high lipophilicity of such systems. In disordered POPC bilayers (L_d phase), the label reports a more polar environment, while increasing the bilayer unsaturation (DOPC) leads to a greater red shift, probably due to the greater bilayer polarity. Sphingomyelin/cholesterol membranes (L_o phase) show intermediate values between $L_{\beta'}$ and L_d. An increase in the fractional content of negatively charged lipids also shifts the emission band of the dye to the red. The most polar environment was observed with DOPG membranes, which combine the flexibility of unsaturated fatty acid chains and negative charge. The observed emission band shifts correlate with the bilayer polarity, as is also indirectly supported by a decrease in the fluorescence emission quantum yields from ~24 to ~8%, in parallel with the red shift. This type of sensitivity complements the lipid phase dependence displayed by the classical membrane probes.[40]

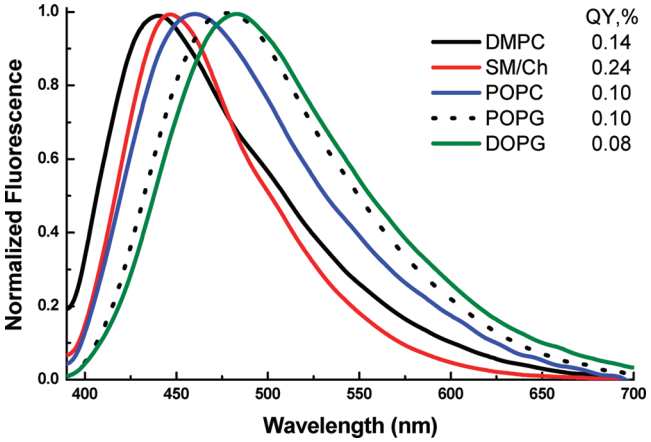


Figure 4. Normalized fluorescence emission spectra of **7AHC** in liposomes of different composition. Spectra were recorded at 20 °C. Probe and lipid concentrations were 2 μM and 0.4 mM, respectively. SM/Ch is a 2:1 sphingomyelin/cholesterol mixture. Excitation at 370 nm.

In conclusion, the introduction of a 7-aryl substituent in 3HC generates a changed orientation of the dipole moment of the fluorophore, leading to the combination of small size and outstanding solvatochromic properties. The new probes have very low tendency for excited-state proton transfer compared with 2-aryl analogues, although the emission peak shows a solvatochromism of up to 180 nm in aprotic solvents. Because of their strong solvatochromism, large Stokes shift, and very low fluorescence in water, **7AHC** and **7AMC** are very useful dyes for spectroscopic studies of turbid solutions, for example, cell suspensions. In addition, methylation of the 3-hydroxy group significantly improves the photostability of the fluorophore. A further, notable feature (advantage) of the new probes in biological applications is the extremely low fluorescence quantum yield (<0.01) in polar protic solvents such as water, leading to a significantly reduced background signal and thus creating the effect of an on−off intensity switch.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis protocols, experimental procedure, dipole moment calculations, solvent dependence, time-resolved measurements, photostability determination, and liposomes. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: luciana.giordano@mpibpc.mpg.de.

Notes

The authors declare no competing financial interest.

[#]Deceased on September 29, 2011.

■ ACKNOWLEDGMENTS

Author Elizabeth A. Jares-Erijman tragically died during the preparation of this manuscript. Her personal and scientific contributions to the study were numerous and essential. We acknowledge the support by the Toxic Proteins Conformation project of the Max Planck Society and by Marie Curie Actions postdoctoral fellowships to V.V.S. We thank the department of Physical Biochemistry (MPI BPC) for access to the time-resolved spectrofluorimeter.

■ REFERENCES

(1) Sinkeldam, R. W.; Greco, N. J.; Tor, Y. Fluorescent Analogs of Biomolecular Building Blocks: Design, Properties, and Applications. *Chem. Rev.* **2010**, *110*, 2579−2619.

(2) Tsien, R. Y. Fluorescence Readouts of Biochemistry in Live Cells and Organisms. In *Molecular Imaging. Principles and Practice*; Weissleder, R., Ross, B. D., Rehemtulla, A.; Gambhir, S. S., Ed.; People's Medical Publishing House (PMPH): Shelton, CT, 2010; pp 808−828.

(3) Spence, R. P. H. M. T. Z. *Handbook of Fluorescent Probes and Research Chemicals*, 11th ed.; Molecular probes: Eugene, OR, 2010.

(4) Loving, G. S.; Sainlos, M.; Imperiali, B. Monitoring Protein Interactions and Dynamics with Solvatochromic Fluorophores. *Trends Biotechnol.* **2010**, *28*, 73−83.

(5) Cohen, B. E.; Pralle, A.; Yao, X.; Swaminath, G.; Gandhi, C. S.; Jan, Y. N.; Kobilka, B. K.; Isacoff, E. Y.; Jan, L. Y. A Fluorescent Probe Designed for Studying Protein Conformational Change. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 965−970.

(6) Reichardt, C. Solvatochromic Dyes as Solvent Polarity Indicators. *Chem. Rev.* **1994**, *94*, 2319−2358.

(7) Marini, A.; Munoz-Losa, A.; Biancardi, A.; Mennucci, B. What Is Solvatochromism? *J. Phys. Chem. B* **2010**, *114*, 17128−17135.

(8) Rezende, M. C.; Dominguez, M.; Aracena, A.; Millan, D. Solvatochromism and Electrophilicity. *Chem. Phys. Lett.* **2011**, *514*, 267−273.

(9) Klymchenko, A. S.; Demchenko, A. R. Multiparametric Probing of Microenvironment with Solvatochromic Fluorescent Dyes. *Methods Enzymol.* **2008**, *450*, 37−58.

(10) Song, S. M.; Ju, D.; Li, J. F.; Li, D. X.; Wei, Y. L.; Dong, C. A.; Lin, P. H.; Shuang, S. M. Synthesis and Spectral Characteristics of Two Novel Intramolecular Charge Transfer Fluorescent Dyes. *Talanta* **2009**, *77*, 1707−1714.

(11) Filarowski, A.; Kluba, M.; Cieslik-Boczula, K.; Koll, A.; Kochel, A.; Pandey, L.; De Borggraeve, W. M.; Van der Auweraer, M.; Catalan, J.; Boens, N. Generalized Solvent Scales as a Tool for Investigating Solvent Dependence of Spectroscopic and Kinetic Parameters. Application to Fluorescent Bodipy Dyes. *Photochem. Photobiol. Sci.* **2010**, *9*, 996−1008.
(12) Weber, G.; Farris, F. J. Synthesis and Spectral Properties of a Hydrophobic Fluorescent-Probe — 6-Propionyl-2-(Dimethylamino)-Naphthalene. *Biochemistry* **1979**, *18*, 3075−3078.
(13) Samanta, A.; Fessenden, R. W. Excited State Dipole Moment of Prodan as Determined from Transient Dielectric Loss Measurements. *J. Phys. Chem. A* **2000**, *104*, 8972−8975.
(14) Kucherak, O. A.; Didier, P.; Mely, Y.; Klymchenko, A. S. Fluorene Analogues of Prodan with Superior Fluorescence Brightness and Solvatochromism. *J. Phys. Chem. Lett.* **2010**, *1*, 616−620.
(15) Everett, R. K.; Nguyen, A. A.; Abelt, C. J. Does Prodan Possess an O-TICT Excited State? Synthesis and Properties of Two Constrained Derivatives. *J. Phys. Chem. A* **2010**, *114*, 4946−4950.
(16) Cwiklik, L.; Aquino, A. J. A.; Vazdar, M.; Jurkiewicz, P.; Pittner, J.; Hof, M.; Lischka, H. Absorption and Fluorescence of Prodan in Phospholipid Bilayers: A Combined Quantum Mechanics and Classical Molecular Dynamics Study. *J. Phys. Chem. A* **2011**, *115*, 11428−11437.
(17) Kotaka, H.; Konishi, G.; Mizuno, K. Synthesis and Photoluminescence Properties of π-Extended Fluorene Derivatives: The First Example of a Fluorescent Solvatochromic Nitro-Group-Containing Dye with a High Fluorescence Quantum Yield. *Tetrahedron Lett.* **2010**, *51*, 181−184.
(18) Lu, Z.; Lord, S. J.; Wang, H.; Moerner, W. E.; Twieg, R. J. Long-Wavelength Analogue of Prodan: Synthesis and Properties of Anthradan, a Fluorophore with a 2,6-Donor−Acceptor Anthracene Structure. *J. Org. Chem.* **2006**, *71*, 9651.
(19) Vazquez, M. E.; Blanco, J. B.; Imperiali, B. Photophysics and Biological Applications of the Environment-Sensitive Fluorophore 6-*N*,*N*-Dimethylamino-2,3-naphthalimide. *J. Am. Chem. Soc.* **2005**, *127*, 1300−1306.
(20) Baathulaa, K.; Xu, Y. F.; Qian, X. H. Unusual Large Stokes Shift and Solvatochromic Fluorophore Synthesis, Spectra, and Solvent Effect of 6-Substituted 2,3-Naphthalimide. *J. Photochem. Photobiol., A* **2010**, *216*, 24−34.
(21) Loving, G.; Imperiali, B. A Versatile Amino Acid Analogue of the Solvatochromic Fluorophore 4-*N*,*N*-Dimethylamino-1,8-naphthalimide: A Powerful Tool for the Study of Dynamic Protein Interactions. *J. Am. Chem. Soc.* **2008**, *130*, 13630−13638.
(22) Fakhari, A.; Rokita, S. E. A New Solvatochromic Fluorophore for Exploring Nonpolar Environments Created by Biopolymers. *Chem. Commun.* **2011**, *47*, 4222−4224.
(23) M'Baye, G.; Klymchenko, A. S.; Yushchenko, D. A.; Shvadchak, V. V.; Ozturk, T.; Mely, Y.; Duportail, G. Fluorescent Dyes Undergoing Intramolecular Proton Transfer with Improved Sensitivity to Surface Charge in Lipid Bilayers. *Photochem. Photobiol. Sci.* **2007**, *6*, 71−76.
(24) Klymchenko, A. S.; Pivovarenko, V. G.; Ozturk, T.; Demchenko, A. P. Modulation of the Solvent-Dependent Dual Emission in 3-Hydroxychromones by Substituents. *New J. Chem.* **2003**, *27*, 1336−1343.
(25) Chou, P. T.; Huang, C. H.; Pu, S. C.; Cheng, Y. M.; Liu, Y. H.; Wang, Y.; Chen, C. T. Tuning Excited-State Charge/Proton Transfer Coupled Reaction Via the Dipolar Functionality. *J. Phys. Chem. A* **2004**, *108*, 6452−6454.
(26) Klymchenko, A. S.; Mely, Y. 7-(2-Methoxycarbonylvinyl)-3-hydroxychromones: New Dyes with Red Shifted Dual Emission. *Tetrahedron Lett.* **2004**, *45*, 8391−8394.
(27) Klymchenko, A. S.; Yushchenko, D. A.; Mely, Y. Tuning Excited State Intramolecular Proton Transfer in 3-Hydroxyflavone Derivative by Reaction of Its Isothiocyanate Group with an Amine. *J. Photochem. Photobiol., A* **2007**, *192*, 93−97.
(28) Brucker, G. A.; Kelley, D. F. Role of Phenyl Torsion in the Excited-State Dynamics of 3-Hydroxyflavone. *J. Phys. Chem.* **1988**, *92*, 3805−3809.
(29) Soujanya, T.; Fessenden, R. W.; Samanta, A. Role of Nonfluorescent Twisted Intramolecular Charge Transfer State on the Photophysical Behavior of Aminophthalimide Dyes. *J. Phys. Chem.* **1996**, *100*, 3507−3512.
(30) Mes, G. F.; Dejong, B.; Vanramesdonk, H. J.; Verhoeven, J. W.; Warman, J. M.; Dehaas, M. P.; Horsmanvandendool, L. E. W. Excited-State Dipole-Moment and Solvatochromism of Highly Fluorescent Rod-Shaped Bichromophoric Molecules. *J. Am. Chem. Soc.* **1984**, *106*, 6524−6528.
(31) Kucherak, O. A.; Richert, L.; Mely, Y.; Klymchenko, A. S. Dipolar 3-Methoxychromones as Bright and Highly Solvatochromic Fluorescent Dyes. *Phys. Chem. Chem. Phys.* **2012**, *14*, 2292−2300.
(32) Furstenberg, A.; Vauthey, E. Excited-State Dynamics of the Fluorescent Probe Lucifer Yellow in Liquid Solutions and in Heterogeneous Media. *Photoch. Photobio. Sci.* **2005**, *4*, 260−267.
(33) Il'ichev, Y. V.; Kuhnle, W.; Zachariasse, K. A. Intramolecular Charge Transfer in Dual Fluorescent 4-(Dialkylamino)benzonitriles. Reaction Efficiency Enhancement by Increasing the Size of the Amino and Benzonitrile Subunits by Alkyl Substituents. *J. Phys. Chem. A* **1998**, *102*, 5670−5680.
(34) Lord, S. J.; Lu, Z. K.; Wang, H.; Willetst, K. A.; Schuck, P. J.; Lee, H. L. D.; Nishimura, S. Y.; Twieg, R. J.; Moerner, W. E. Photophysical Properties of Acene DCDHF Fluorophores: Long-Wavelength Single-Molecule Emitters Designed for Cellular Imaging. *J. Phys. Chem. A* **2007**, *111*, 8934−8941.
(35) Fery-Forgues, S.; Fayet, J. P.; Lopez, A. Drastic Changes in the Fluorescence Properties of NBD Probes with the Polarity of the Medium — Involvement of a TICT State. *J. Photochem. Photobiol., A* **1993**, *70*, 229−243.
(36) Ghanadzadeh Gilani, A.; Moghadam, M.; Zakerhamidi, M. S. Solvatochromism of Nile Red in Anisotropic Media. *Dyes Pigm.* **2012**, *92*, 1052−1057.
(37) Dimroth, K.; Bohlmann, F.; Reichard, C.; Siepmann, T. Über Pyridinium-N-Phenol-Betaine und Ihre Verwendung Zur Charakterisierung Der Polarität Von Lösungsmitteln. *Liebigs Ann. Chem.* **1963**, *661*, 1−37.
(38) Catalan, J. Toward a Generalized Treatment of the Solvent Effect Based on Four Empirical Scales: Dipolarity (SdP, a New Scale), Polarizability (SP), Acidity (SA), and Basicity (SB) of the Medium. *J. Phys. Chem. B* **2009**, *113*, 5951−5960.
(39) Shynkar, V. V.; Mely, Y.; Duportail, G.; Piemont, E.; Klymchenko, A. S.; Demchenko, A. P. Picosecond Time-Resolved Fluorescence Studies Are Consistent with Reversible Excited-State Intramolecular Proton Transfer in 4′-(Dialkylamino)-3-hydroxyflavones. *J. Phys. Chem. A* **2003**, *107*, 9522−9529.
(40) Demchenko, A. P.; Mely, Y.; Duportail, G.; Klymchenko, A. S. Monitoring Biophysical Properties of Lipid Membranes by Environment-Sensitive Fluorescent Probes. *Biophys. J.* **2009**, *96*, 3461.