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# Reversible Synthesis and Characterization of Dynamic Imino Analogues of Trimethine and Pentamethine Cyanine Dyes

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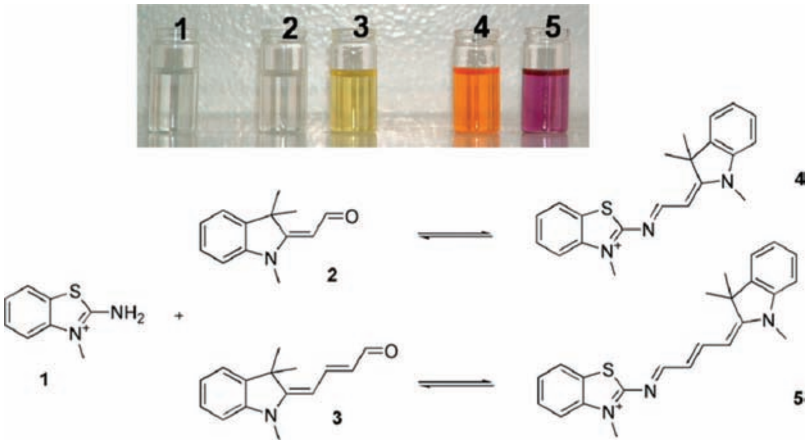
## ABSTRACT



**A new family of unsymmetrical imine-based trimethine and pentamethine cyanine dye analogues is reported that can form under reversible and thermodynamically controlled conditions from non- or weakly emissive amine and aldehyde building blocks. These dynamic fluorophores show spectroscopic properties comparable to those of their parent cyanine dyes and are responsive to external effectors.**

Cyanine dyes generically consist of a conjugated system based on a polymethine chain linking two nitrogen-containing heterocycles (e.g., indoles, benzothiazoles).[1] They are generally named based on the number of carbon atoms in the polymethine chain. Trimethine and pentamethine dyes exhibit absorption maxima at 550 and 650 nm, respectively, and emission maxima around 570 and 670 nm, respectively, in the green or red part of the spectrum. Due to their relative chemical stability, high molar absorption coefficient, and high fluorescence quantum yield, cyanine dyes have been extensively used in photography,[2] optical data storage,[3] and more recently, proteomics[4] and biomolecular labeling.[5] Of particular interest are molecules that can reversibly switch between a nonemissive state and an emissive state. Most representative examples are fluorogenic unsymmetrical cyanine dyes which become fluorescent upon interaction with

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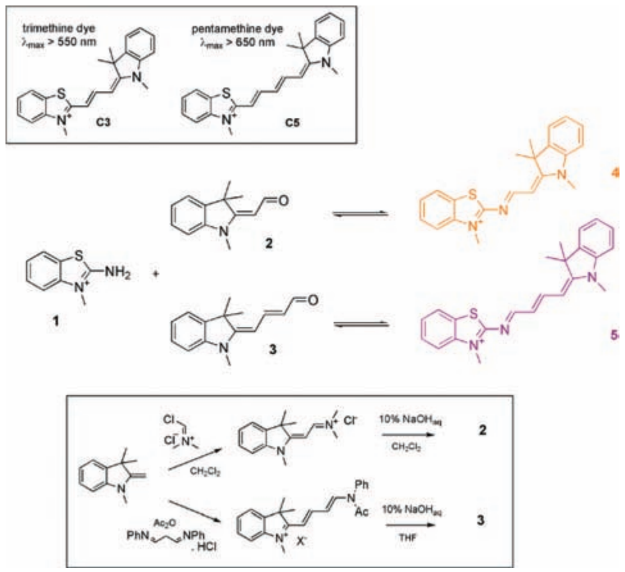
specific nucleic acids[6] or proteins[7] and photochromic compounds[8] which can undergo a reversible change of their optical properties under illumination. Such dynamic systems can find valuable applications as fluorescent supramolecular devices, in particular for the design of smart materials.[9] Herein, we introduce a new family of dynamic fluorophore analogues of the well-known cyanine dyes (from the trimethine and pentamethine family) that can be obtained via a reversible and covalent reaction between non- and/or weakly fluorescent benzothiazolium amine and indolenin aldehyde building blocks (Scheme 1). These cyanine dye analogues differ from the original dyes solely by the introduction of an imine bond into the polymethine chain, thus making their formation reversible and adaptive to the pressure of external conditions.

**Scheme 1.** General Structures of Unsymmetrical Trimethine and Pentamethine Cyanine Dyes (**C3** and **C5**). Proposed Synthetic Strategies for Their Imine Analogues **4** and **5** and Synthetic Route for Aldehydes **2** and **3**



As a model system, the reactions between an *N*-methyl-2-amino benzothiazole and two functionalized indolenin aldehydes were investigated. Amine **1** was obtained by quarternization of commercially available 2-aminobenzothiazole with methyl iodide. Aldehydes **2** and **3**[10] were synthesized by reaction of 1,2,3,3-tetramethylindolenin with (chloromethylene)dimethyl ammonium chloride and malonaldehyde bisphenylimine hydrochloride, respectively, followed by alkaline hydrolysis of the activated hemicyanine intermediate. In order to monitor the reaction between amine **1** and Fisher's base aldehyde **2**, a 10 mM stoichiometric mixture of both building blocks was heated (50 °C) in DMSO-$d_6$, and $^1$H NMR spectra were recorded every 24 h for 3 days. A new set of signals slowly appeared at the expense of the signals of monomers **1** and **2** along with a pale orange coloration of the solution. Particularly striking was the appearance of two doublets at 8.52 and 6.50 ppm that could be assigned to the imino proton and the adjacent methine proton, respectively, of the predicted C3 analogue **4** (Figure 1a). The intensity of these signals slowly increased for 3 days until saturation was reached.

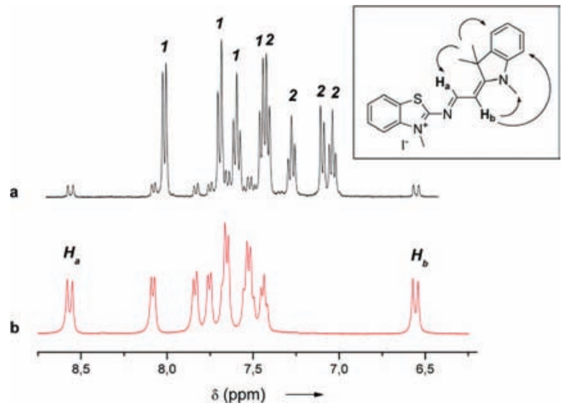


**Figure 1.** $^1$H NMR spectra of (a) a stoichiometric mixture of amine **1** and aldehyde **2** after reacting for 72 h at 50 °C in DMSO-$d_6$ and (b) isolated C3 imine analogue **4**. The all-trans structure of **4** is represented in the inset (arrows indicate the observed NOESY correlations).

In order to unambiguously characterize this new species, a mixture of amine **1** and aldehyde **2** in a mixture of toluene and DMF was heated at 95 °C in a Dean−Stark apparatus for 3 days. The imine derivative **4** was obtained pure as a bright orange solid and was subsequently fully characterized. The $^1$H NMR of the isolated imine (Figure 1b) was consistent with the new set of signals appearing when mixing **1** and **2**. This allowed us to estimate around 15% the maximal conversion of aldehyde and amine into the corresponding imine at 50 °C. It is noteworthy that despite the possible cis/trans isomerization of the imine bond formed, only one isomer was always observed by $^1$H and $^{13}$C NMR. The all-trans conformation of the polymethine/imine chain was assigned by NOESY, thus confirming that this imino dye adopts the same thermodynamically stable conformation as a trimethine cyanine dye.

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(10) The formation of aldehyde **3** as a C5 byproduct resulting from the hydrolysis of immobilized hemicyanines was recently proposed based on M.S. analysis in: Mason, S. J.; Hake, J. L.; Nairne, J.; Cummins, W. J.; Balasubramanian, S. *J. Org. Chem.* **2005**, *70*, 2939.

Next, $^1$H NMR experiments were carried out from amine **1** and aldehyde **3** under similar conditions as with aldehyde **2**. As described above for imine **4**, the slow formation of imine **5** was evidenced by $^1$H NMR. Compound **5** was isolated as a dark purple solid and was also present in solution as a unique all-trans isomer (Supporting Information). It is noteworthy that both imino dyes **4** and **5** proved highly stable in their solid state while slowly regenerating the amine and aldehyde precursors when stored in solution for more than 1 h. Fresh solutions of analytically pure samples of compounds **4** and **5** and of their aldehyde precursors **2** and **3** were prepared in DMSO, and their spectroscopic properties were investigated using UV−vis and fluorescence (Table 1).

**Table 1.** Spectral Properties of the Imino Dyes **4** and **5** and of Their Aldehyde Precursors **2** and **3**[a]

| compd | $\lambda_{abs}$ (nm) | $\varepsilon$ ($M^{-1}$ $cm^{-1}$) | $\lambda_{em}$ (nm) | QY[b] |
|---|---|---|---|---|
| **2** | 338 | $5.41 \times 10^4$ | 410 | 0.00017 |
| **3** | 410 | $5.47 \times 10^4$ | 460 | 0.006 |
| **4** | 469 | $7.42 \times 10^4$ | 522 | 0.010 |
| **5** | 579 | $4.83 \times 10^4$ | 608 | 0.033 |
| **C3**[11] | 547 | $5.70 \times 10^4$ | 572 | 0.016 |
| **C5**[11] | 648 | $8.82 \times 10^4$ | 674 | 0.038 |

[a] All measurements were taken in DMSO, and reported values are the average of at least two independent measurements. [b] Fluorescence quantum yields were determined by comparative method using quinine sulfate, fluorescein, and sulforhodamine 101 as reference standards.

In comparison to their parent C3 and C5 cyanine dyes (i.e., lacking the imine bond), imines **4** and **5** have their maximum emission of fluorescence at slightly shorter wavelengths (−50 and −68 nm, respectively). However, the introduction of an imine bond into the conjugated polymethine chain has only a very limited effect on either the Stokes' shift or the molar absorption coefficient of the dye. Although notably low (0.01−0.033), fluorescence quantum yields of both imines proved significantly higher than those of their corresponding aldehyde precursors and only slightly lower than those of commonly used trimethine and pentamethine dyes. It is also noteworthy that the absorption spectra of aldehydes **2** and **3** do not overlap with those of their corresponding imines. Therefore, when forming imine **4** (or **5**) from amine **1** and aldehyde **2** (or **3**), excitation of the reaction mixture at 470 nm (or 580 nm) enables the specific detection of the fluorescence emitted by the imino dye only. Of particular interest is the case of bright orange and fluorescent C3 imino analogue **4** which results from the reaction between a dark and colorless amine **1** and a colorless and minimally fluorescent aldehyde **2** (fluorescence quantum yield 60-fold lower than that of imine **4**).

To elucidate the changes in electronic structure upon introduction of an imino group into the polymethine chain of cyanine dyes we performed a time-dependent density functional theory (TD-DFT)[12] study. Calculations were carried out for compounds **4** and **5** as well as for reference compounds C3 and C5 on the B3LYP/6-311G(d)//B3LYP/6-31G(d) level of theory using an implicit treatment of the solvent (DMSO). The scaling scheme of Champagne et al.[13] was used to correct for the slight, intrinsic overestimation of excitation energies by the TD-DFT method. The calculated energies for vertical excitation of 1.89, 2.06, 2.23, and 2.42 eV for dyes C5, **5**, C3, and **4** are in good agreement with the experimental values of 1.91, 2.14, 2.26, and 2.64 eV. The experimental shift to larger excitation energies, i.e., shorter wavelengths upon introduction of an imino group into the polymethine chain, is also seen in the calculations: 44 nm for **4** versus C3 and 54 nm for **5** versus C5. While the energy-minimized structures of C3 and C5 and of their respective imino analogues **4** and **5** all proved perfectly planar, the shift in wavelength can be understood in terms of the larger HOMO−LUMO splitting in the ground state for imino compounds **4** and **5** in comparison to the parent C3 and C5 molecules, respectively (Figure 2; note that the HOMO−LUMO gap in the ground state does not correspond to the reported values of the excitation energy as determined from TD-DFT). Due to the positive charge of the system, both HOMO and LUMO are largely negative in energy. It is not surprising that the orbitals of the imino dye **4** are shifted to lower energy with respect to the all-methine dye, the electrons being more strongly bound in **4** than in C3 due

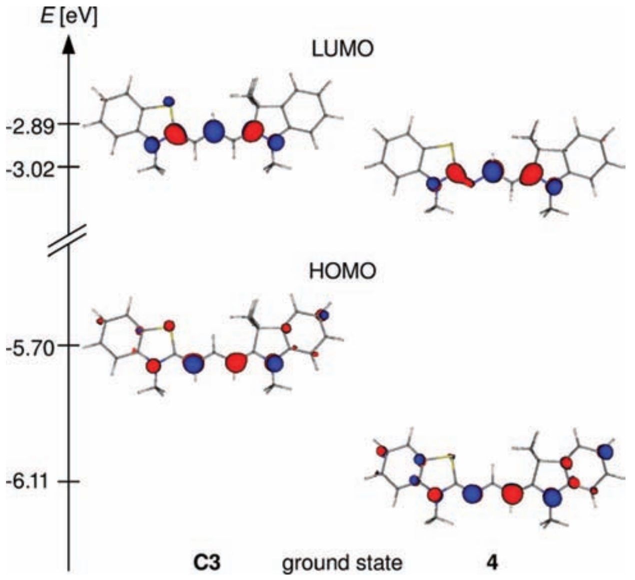

**Figure 2.** Highest occupied molecular orbital (HOMO) and lowest unoccupied orbital molecular orbital (LUMO) of C3 and **4**. The isodensity surfaces of the orbitals were drawn with the program MOLEKEL 5.3[14] from GAUSSIAN 03[15] cube-files with a contour value of ±0.06.

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(11) For direct comparison, analytically pure samples of the C3 and C5 direct analogues of imines **4** and **5** were prepared according to the literature procedure, and their spectroscopic properties in DMSO were determined under identical conditions. See the Supporting Information.

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to the larger electronegativity of the nitrogen. The HOMO is also more strongly delocalized over the entire molecule in **4** than in C3, i.e., the atomic orbital coefficients of the heterocycles are larger in **4** than in C3. The replacement of a methine group by an imino group leads to a better overlap between the polymethine chain and the nitrogen-containing heterocycles, and thus, the HOMO is more stabilized. On the other hand, the LUMO is slightly less delocalized in **4** than in C3. The orbital coefficients of the sulfur atom are smaller in **4** than in C3, whereas for the imino nitrogen the opposite is true. As a result of the larger delocalization (stabilization) of the HOMO with respect to the LUMO in **4** than in C3 the gap between these orbitals is larger for the former. This explains qualitatively the larger excitation energy and the shorter absorbance wavelength for the imino dyes **4** and **5** in comparison to C3 and C5, respectively.

Finally, we investigated whether the reaction of formation of cyanine dye imino analogues from amine and aldehyde building blocks was truly reversible. The dynamic system could then undergo constitutional reorganization in response to an external stimulus.[16] A stoichiometric mixture of amine **1** and aldehyde **2** (25 mM each) in DMSO was stirred at room temperature until thermodynamic equilibrium was reached (48 h). The perturbation of the system was monitored by HPLC, UV−vis, and fluorescence spectroscopy. At equilibrium, only a very small amount of C3 imine **4** was formed. Despite this low level of conversion, imine **4** was easily detected by fluorescence spectroscopy and could also be visualized by UV−vis (yellow color). The mixture was then heated to 60 °C and was again allowed to reach equilibrium. Equilibrium was reached after 24 h which was different from that observed at room temperature. The absolute amount of C3 analogue **4** present at equilibrium was increased by about 5-fold (as measured by both HPLC and fluorescence) (Figure 3). When the reaction mixture was being cooled to room temperature, the system reorganized again until it reached an equilibrium position (after 72 h) that was comparable to that obtained initially before heating, thus proving true reversibility of the system. Similar results were obtained when reacting amine **1** (50 mM) with both aldehydes **2** and **3** (25 mM each) simultaneously.

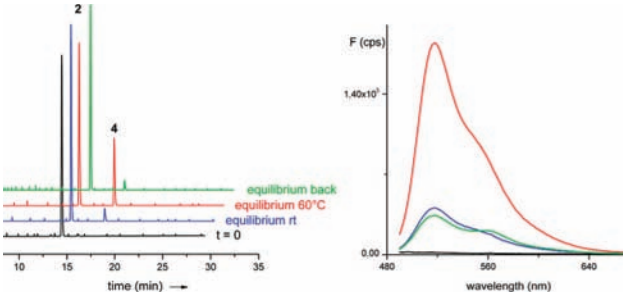

**Figure 3.** HPLC traces and fluorescence spectra showing the formation of imine **4** from a mixture of amine **1** and aldehyde **2** (25 mM each). Starting position (black) and positions of equilibrium after 48 h at rt (blue), then after heating 24 h at 60 °C (red), and after cooling back for 72 h at rt (green) are represented. HPLC traces show the absolute ratios of aldehyde **2** (retention time 14.5 min) and imine **4** (retention time 18.5 min). Fluorescence emission spectra were recorded when exciting at 480 nm.

It is also noteworthy that reorganization of the equilibrating mixture can be monitored in real time by fluorescence spectroscopy. While this dynamic system takes 48 h to equilibrate at room temperature, less than 24 h is required to reach equilibrium when the reaction is carried out at 60 °C (see the Supporting Information for kinetics of imino dye formation).

Synthesis of cyanine dyes is generally accomplished by the irreversible stepwise reaction between nucleophilic heterocycles (e.g., 1,2,3,3-tetramethylindolenin) and a polyene-chain precursor such as an amidine and proceeds via the formation of a hemicyanine intermediate. Herein, we reported the first family of cyanine dye (trimethine and pentamethine) imine analogues that are easily accessible via a reversible and thermodynamically controlled reaction from readily available amine and aldehyde building blocks. We provided proof-of-concept that this dynamic system could reorganize in response to an external stimulus, thus leading to a measurable perturbation of the global UV−vis and fluorescence spectra of the equilibrating mixture. The development of smart materials based on this concept is currently underway in our laboratory.

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**Supporting Information Available:** General experimental procedures and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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