Near-Infrared Fluorophores

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Bright Near-Infrared Fluorophores Based on Squaraines by Unexpected Halogen Effects**

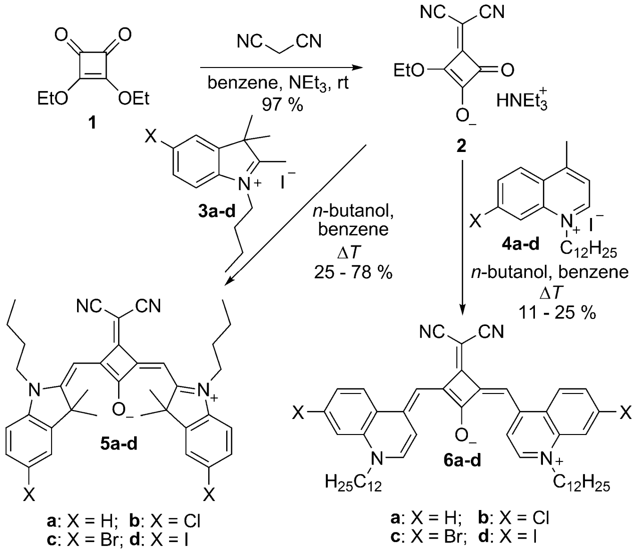
*Ulrich Mayerhöffer, Benjamin Fimmel, and Frank Würthner**

Dedicated to Professor Klaus Müllen on the occasion of his 65th birthday

Near-infrared (NIR) absorbing dyes with absorption above 700 nm are attracting increasingly more interest of a vast scientific community as they open unique opportunities for applications in the fields spanning from materials science to biology and medicine.[1] In respect to materials science such NIR chromophores may facilitate the manufacturing of transparent devices that are highly desirable since more than 50% of the solar energy reaches the earth in the NIR region, and indeed intensive efforts are continuing in the design of novel photovoltaic active NIR materials.[2] An even broader application scope exists for such chromophores that not only absorb, but also emit light in the NIR region. Besides their high significance for photophysical technologies, NIR fluorophores are of enormous importance to specifically label products such as oil or code banknotes, as well as for biological and medicinal applications.[3] The NIR spectral region is often referred to as the optical window in biological tissues because autoabsorption and autofluorescence of biomolecules is least between 650 and 1200 nm combined with low light scattering and deep tissue penetration of NIR radiation.[4] Thus, NIR fluorophores provide a benign and non-invasive tool for bioimaging with substantially increased sensitivity compared to ultraviolet (UV) or visible (Vis) fluorophoric probes.

The suitability of fluorophores for the above-mentioned applications is determined by their chemical and photophysical properties. The former addresses stability and reactivity of the chromophore within its application environment, whereas the latter feature approaches its contrast strength. The decisive criterion that allows an easy evaluation of the quality of a fluorophore is provided by its brightness, the latter being proportional to the product of the molar absorption coefficient ($\varepsilon_{\max}$) and the quantum yield (Φ_{fl}), and thus reflecting both the amount of photons absorbed and the efficiency with which those photons are emitted by the fluorophore. An ideal fluorophore should, therefore, feature sharp and intense absorption spectra combined with high fluorescence quantum yield. Because of their inherent low band gaps, NIR materials often suffer from a dominant vibronic coupling between ground and excited state which promotes nonradiative deactivation pathways. As a result, most NIR materials are nonfluorescent or show low fluorescence efficiency. Whilst numerous examples of bright fluorophores with high quantum yields in the visible region are known, reports on efficient NIR fluorophores are rather scarce. Among the currently known brightest fluorophores with emission in the region above 700 nm are boron-dipyrromethenes (BODIPYs) and pyrrolopyrrole cyanines with emission maxima up to 780 and 966 nm, respectively.[5] Squaraine dyes are among the most promising candidates for NIR fluorophores because they show sharp and intense absorption in the long-wavelength region, and because of their rigid structure they possess good photostability.[6] Indeed, squaraines have already been applied in bioimaging,[7] and as sensitizers, therapeutic agents, and sensors for biological applications.[8] However, the absorption and emission wavelengths of the squaraine chromophores applied so far are located in the region between 600–750 nm. Because of this limited wavelength range, the known squaraine-based molecular probes can only cover a small range of the biological optical window.

In the course of our search on squaraine-based photovoltaic materials,[2c] we observed in a series of squaraine dyes (**5a**–**d** in Scheme 1) an unexpected halogen effect that led to a



Scheme 1. Two-step synthesis of squaraines **5a**–**d** and **6a**–**d** (rt = room temperature).

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[*] U. Mayerhöffer, B. Fimmel, Prof. Dr. F. Würthner
Universität Würzburg, Institut für Organische Chemie
and Röntgen Research Center for Complex Material Systems
Am Hubland, 97074 Würzburg (Germany)
E-mail: wuerthner@chemie.uni-wuerzburg.de

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bathochromic shift of absorption, accompanied by an increase of the molar absorption coefficient and an amazing increase of the fluorescence quantum yield in the series **5a** (H) < **5b** (Cl) < **5c** (Br) < **5d** (I).[9] This observation is intriguing as the common perception suggests a fluorescence quenching effect of heavy atoms like bromine and iodine because of a perturbation of the fluorescing S_1 state by spin–orbit coupling.[10] To utilize the observed unusual halogen effect, we have designed the squaraine-based NIR fluorophores **6a–d** (Scheme 1) which show excellent fluorescence properties with emission maxima up to 922 nm.

The synthetic route for squaraine NIR fluorophores is depicted in Scheme 1. Starting with the squaric acid diethylester **1**, a condensation reaction of the former with malononitrile afforded the dicyanovinyl substituted squaric acid derivative **2**.[11] Because of the electron withdrawing character of the dicyanovinyl unit, the squaric acid derivative **2** displays a higher reactivity towards nucleophilic methylene bases like **3a–d** and **4a–d** compared to that of the respective squaric acid and its diethylester **1**, and hence allowing the hitherto elusive introduction of 4-methylquinolinium iodides **4a–d** as donor units to the squaraine core. Such donors are known to afford the undesired 1,2-condensation product if reacted with squaric acid.[12] The final condensation reaction was performed by refluxing **2** with two equivalents of the respective methylene base (**3a–d** and **4a–d**) in a 1:1 mixture of benzene and *n*-butanol to afford the desired NIR dyes **5a–d** and **6a–d** in 11% to 78% yields.[13]

All newly synthesized squaraines show a main absorption band in the long wavelength region reflecting the band gap of the S_1 transition and an additional, less intense absorption band in the blue region that can be ascribed to a higher S_2 transition (Figure 1). Whilst compounds **5a–d** show absorption close to the NIR region between 683 and 699 nm, the introduction of the 4-methylquinolinum donors **4a–d** leads to a significant bathochromic shift of the absorption to 870 nm for **6a** and up to 900 nm for **6d**.

This bathochromic shift for the series **6a–d** compared to the series **5a–d** of about 200 nm can be ascribed to an increase of the conjugation length. Squaraines can be considered, to some respects, as a special kind of cyanine dyes. Like the latter, squaraines show an odd number of carbon atoms between the two nitrogen atoms fencing the conjugation along the polymethine chain. Because of the quinoline donor moieties in **6a–d**, the number of carbons along this chain increases from 7 in **5a–d** to 11 in **6a–d** and thus reflects the same relation as found for cyanines, which show a bathochromic shift of about 100 nm for each additional dimethine unit within the chain.[14] The optical properties of the squaraines **5** and **6** are summerized in Table 1.

Table 1: Spectral properties of squaraines **5a–d** and **6a–d** in CH_2Cl_2.

Dye	λ_{max} [nm]	ε [$M^{-1}cm^{-1}$]	λ_{em} [nm]	Φ_{em}	$\varepsilon\Phi_{em}$[c] [$M^{-1}cm^{-1}$]
5a[a]	683	195 000	698	0.37 ± 0.02	72 150
5b[a]	694	215 000	708	0.47 ± 0.02	101 500
5c[a]	695	216 000	710	0.47 ± 0.02	101 520
5d[b]	699	221 000	713	0.58 ± 0.02	128 180
6a[b]	870	205 000	890	0.10 ± 0.01	20 500
6b[b]	885	258 000	913	0.11 ± 0.01	28 380
6c[b]	891	251 000	916	0.12 ± 0.01	30 120
6d[b]	900	274 000	922	0.17 ± 0.01	46 580

[a] Fluorescence standard: rhodamine 800 in ethanol; $\Phi_{fl} = 0.21$.
[b] Fluorescence standard: indocyanine green in dimethylsulfoxide (DMSO); $\Phi_{fl} = 0.13$.[15] [c] The product $\varepsilon\Phi_{em}$ is proportional to the optical brightness of the fluorophore, and thus a measure for the contrast strength of the respective compounds.

The data shown in Table 1 reveal a clear trend. Thus, the introduction of halogen atoms leads to an increase of the molar absorption coefficient (ε) and a bathochromic shift, which increases in the series from hydrogen (**5a**, **6a**) to iodine (**5d**, **6d**). This trend can nicely be followed for both squaraine series as illustrated in Figure 2.[16] The substitution of hydrogen in **5a** by chlorine and bromine in **5b** and **5c**, respectively, resulted in a bathochromic shift of about 10 nm, which is accompanied by an increase of the molar absorption coefficient along with a significant increase of the fluorescence quantum yield from $\Phi_{em} = 0.37$ to 0.47. A further increase can

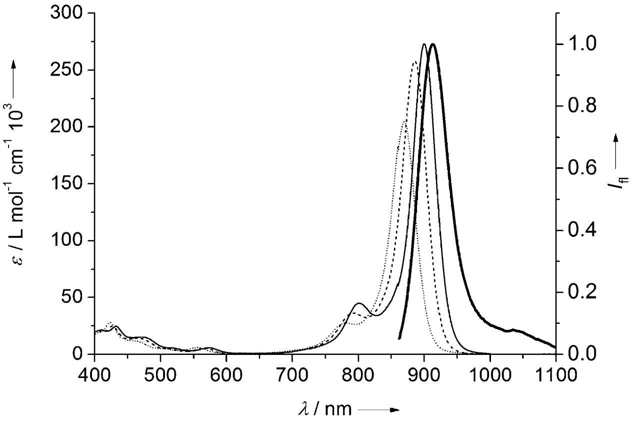

Figure 1. UV/Vis/NIR absorption spectra of squaraines **6a** (dotted line), **6b** (dashed line), and **6d** (thin solid line) in CH_2Cl_2 ($c = 10^{-5}$ M at 25 °C) and the fluorescence spectrum ($\lambda_{ex} = 780$ nm) of squaraine **6d** (thick solid line) in CH_2Cl_2 (at 25 °C).

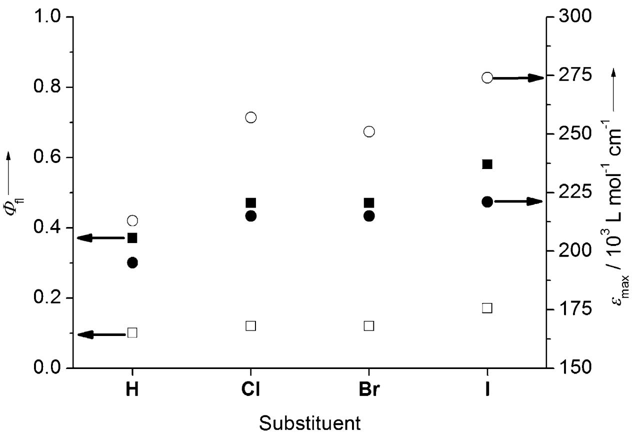

Figure 2. Influence of halogen atoms on the optical properties (squares: Φ_{fl}; circles: ε_{max}) of squaraines **5a–d** (filled symbols) and **6a–d** (open symbols).

be achieved by introduction of iodine in **5d**, leading to a further bathochromic shift of the absorption and emission bands and an increase of extinction to $\varepsilon_{max} = 221\,000\,\text{M}^{-1}\text{cm}^{-1}$. Most notably, the fluorescence quantum yield rises further to $\Phi_{em} = 0.58$, resulting in an amazing optical brightness of about $128\,000\,\text{M}^{-1}\text{cm}^{-1}$. Summarizing these results, the optical brightness of structurally related chromophores can be increased by more than 50% through a simple substitution of hydrogen in **5a** by iodine in **5d**.

This trend can also be observed for the quinoline series **6a–d**. Alike the indolinium series **5a–d**, the introduction of halogen atoms leads to a significant bathochromic shift. All dyes **6a–d** show high molar absorption coefficients of above $200\,000\,\text{M}^{-1}\text{cm}^{-1}$. From the dye **6a** (H) with $\varepsilon_{max} = 205\,000\,\text{M}^{-1}\text{cm}^{-1}$ to **6d** (I) with $\varepsilon_{max} = 274\,000\,\text{M}^{-1}\text{cm}^{-1}$, a drastic increase of the tinctorial strength of more than 30% is observed. Even though the fluorescence quantum yields of **6a–d** are somewhat lower than those of the indolinium donor containing **5a–d**, in regard to their emission wavelength the obtained values are highly creditable as they make them to one of the brightest NIR fluorophores in the wavelength region beyond 900 nm. Starting from **6a** (H), the brightness of the dyes is successively increased by chlorine (**6b**) and bromine (**6c**) to result in a 120% increase for the iodine compound **6d**.

In the light of these results, the question of the origin of this remarkable halogen effect arises. Several previous examples of NIR fluorophores such as BODIPY and pyrrolopyrrole cyanine dyes achieved their NIR fluorescence by complexation of boranes. The complexation leads to a rigidification of the chromophore backbone, resulting in reduced torsional motions and vibronic coupling to the ground state as well as an improved conjugation of the π system and thus an increased tinctorial strength and improved fluorescent properties.

Similar to the above mentioned examples, dicyanovinyl-functionalized squaraines appear to have also a rigid structure as their central four-membered ring confines the π backbone in a *cisoid* conformation. Single-crystal X-ray analyses of all compounds in the series **5a–d** confirm this structural feature and paint a clear picture of coplanarity of the donor–acceptor–donor conjugation (Figure 3, for crystallographic data see the Supporting Information).[17]

The origin of the observed halogen effect in both squaraine series is, nevertheless, not straightforward to explain. On one hand, halogens are expected to have an electron withdrawing effect because of their electronegativity and, on the other hand, they have an electron donating character through a mesomeric +M effect. Quantum chemical DFT calculations show that both of these effects are present in these squaraines.[18] As generally known, the −I effect leads to a decrease of the frontier molecular orbital (FMO) energy levels, which can be proven by cyclic voltammetry (CV) measurements (see the Supporting Information). The +M effect simultaneously leads to a narrowing of the band gap, which is reflected in the observed bathochromic shift of the absorption. The calculated electron density distribution in the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

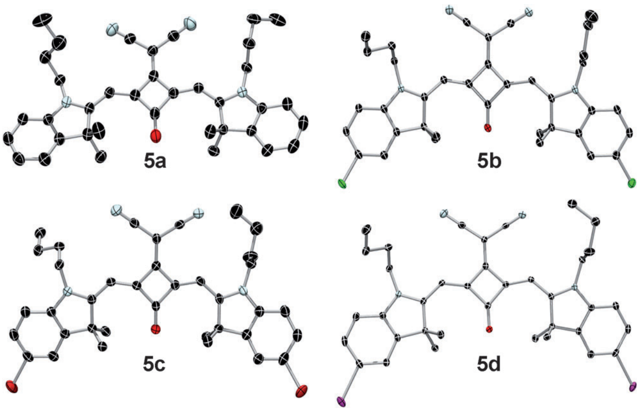

Figure 3. Molecular structures of **5a–d** determined by X-ray analysis. Thermal ellipsoids at the 50% probability level, hydrogen atoms and the disorder of one alkyl chain in **5a** are omitted for clarity.

of **5a** reveals a significant orbital coefficient at the C5 carbon atom of the indolenine donor unit. As can be seen in Figure 4, substitution at this position in **5c** thus leads to an incorporation of the halogen orbitals in the π system of the chromophore. Thereby the orbital coefficients increase with increasing polarizability of the halogen atom (see Figures S10 and S11 in the Supporting Information). Moreover, the +M effect of the halogen atoms can be followed by a transition of electron density from the halogen atom in the HOMO to the chromophore backbone in the LUMO upon excitation, which is exemplarily shown for **5c** in Figure 4. The magnitude of electron density transferred from the halogen increases from fluorine over chlorine to bromine. These results suggest that the halogen atoms strengthen the conjugation along the π system upon excitation, resulting in the observed higher tinctorial strength and the improved fluorescence quantum yields. The same behavior is observed for the NIR compounds **6a–d** as well, leading to the conclusion that the observed halogen effect is due to a transfer of electron density from the halogen substituents to the chromophore core which is more pronounced with increasing polarizability of the halogen substituents.

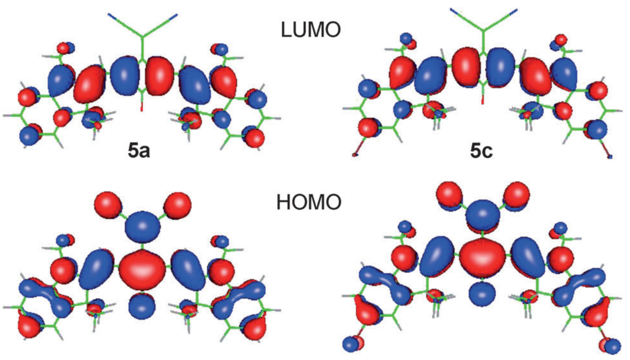

Figure 4. Illustration of the calculated HOMO and LUMO electron density distribution of squaraines **5a** and **5c** (Turbomole V5.10; RI-DFT/B3LYP; basis sets: TZVP/TZV/def-TZVP).

To conclude, we have reported here the synthesis of a series of novel squaraine dyes with unprecedented NIR properties. Furthermore, we could shed light on an unexpected halogen effect that leads, in contrary to common perception about heavy atom quenching effects, to superior fluorescence properties to yield an increase of the optical brightness of the squaraine chromophores in the halogen series from fluorine over chlorine and bromine to iodine. Accordingly, the spectral window for squaraine-based fluorophores could be extended to above 900 nm which opens up new opportunities for NIR imaging and sensorics.

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[16] The substitution of the hydrogen atom in 5-position of **5a** by fluorine (see the Supporting Information) invokes only minor changes, resulting in a slight increase of the molar absorption coefficient from 195 000 to 205 000 $\mathrm{M}^{-1}\mathrm{cm}^{-1}$. The absorption and emission wavelengths are only moderately shifted by 1 nm from 683 to 684 nm. The fluorescence quantum yield slightly reduces from 0.37 to 0.34, resulting in chromophores with an optical brightness of about 70 000 $\mathrm{M}^{-1}\mathrm{cm}^{-1}$ which is comparable with that of **5a**.

[17] CCDC 847682 (**5a**), 847680 (**5b**), 847679 (**5c**), and 847681 (**5d**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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