Synthesis of a Novel Chiral Squaraine Dye and Its Unique Aggregation Behavior in Solution and in Self-Assembled Monolayers**

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Squaraine dyes have been studied extensively in the last few decades due to their advantageous optical properties[1] that enable applications including imaging technologies[1c] and photoconducting devices,[2] photovoltaics[3] and non-linear optics,[4] and sensing[5] and photodynamic therapy.[6] The isolated squaraine chromophore exhibits an intense and narrow absorption band in the long-wavelength range of the visible spectrum. Furthermore, the dye displays a pronounced tendency to form H- and J-type aggregates in different homogenous and heterogeneous media.[7] In order to harness the beneficial opto-electronic characteristics of this unique class of chromophores for the construction of improved devices, control over their aggregation behavior is absolutely critical since it influences electron- and energy-transport processes.[8,9] Since chirality has been used as a guiding element to direct self-assembly processes, we investigated the influence of introducing a chiral bias to squaraine aggregation. Here, we present our initial results on the synthesis of a novel chiral squaraine dye[10] and the investigation of its unique aggregation behavior in aqueous solution and on a solid substrate.

Condensation of electron-rich aromatics with squaric acid in an azeotropic solvent mixture such as toluene/1-butanol provides a simple access to squaraines.[11] Highly activated *N,N*-dialkylated 3,5-dihydroxyanilines are conveniently generated in situ by condensation of secondary amines with 1,3,5-trihydroxybenzene (phloroglucinol),[12] and subsequent reaction with squaric acid in the same pot leads to formation of bis(4-dialkylamino-2,6-dihydroxyphenyl)squaraines, which offer the advantages of much higher intrinsic fluorescence quantum yields,[1a] increased relative chemical stability,[7g,13] and stronger aggregation tendency[1b,7d,14] than their non-hydroxylated analogues. Such a two-step, one-pot procedure[12] can also be applied to the synthesis of polysquaraines, useful for cation sensing.[15] Since secondary amines are mandatory to avoid squaramide formation,[11b] the natural amino acid L-proline is an obvious choice for a chiral building block. Preliminary experiments[16] indicated that an ester linkage to the side chain was not stable during the squaraine condensation step due to transesterification and therefore, initial reduction to L-prolinol, and attachment of the side chains via ether linkages was pursued. Our route (Scheme 1) involves preparation of the alkylated L-prolinol followed by the two-step, one-pot procedure to access chiral squaraine **1** carrying nonpolar, linear *n*-alkyl side chains. This synthesis takes advantage of the enhanced nucleophilicity of aromatics possessing cyclic, i.e., pyrrolidino, rather than acyclic secondary amine substituents.[17]
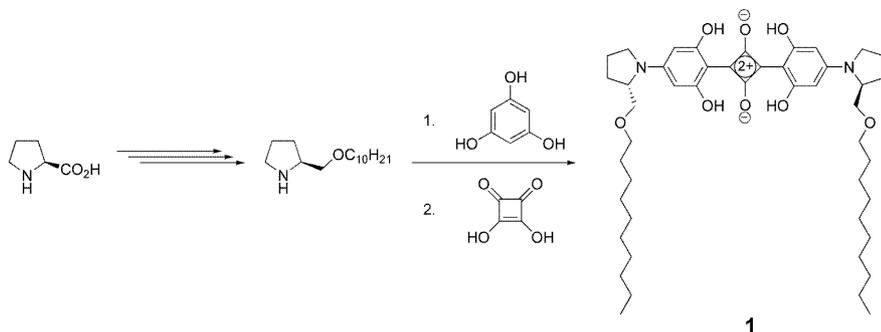
Compound **1** showed a characteristic sharp (full width at half maximum, FWHM ~ 20 nm) and intense (molar absorption $\varepsilon \sim 300\,000$ M^{-1} cm^{-1}) absorption band centered around 640 nm that is indicative of the presence of mostly monomeric, i.e., non-aggregated, squaraine chromophores.[18] Significant variation of solvent polarity, from cyclohexane to chloroform to acetonitrile, led to negligible bathochromic shifts ($637 \rightarrow 638 \rightarrow 642$ nm).[18] Emission measurements revealed sharp fluorescence spectra with relatively small Stokes shifts ($\Delta\lambda_{max} \sim 17$ nm) and high fluorescence quantum yields ($\Phi_f \sim 0.85$), illustrating the rigidified character of the chromophore due to intramolecular hydrogen bonding.[1a]

Due to the hydrophobic nature of the squaraine moiety[1,19] and the unique quality of water to promote aggregation as a consequence of the operating hydrophobic effect, solvent titration experiments involving a systematic variation of the water content in acetonitrile solution were carried out (Fig. 1). The UV-vis spectra (Fig. 1, top) clearly show that aggregation is induced above a critical water concentration of ~ 10 vol.-%. Above this threshold value, the monomer band initially rapidly decreases, and, at ~ 18 vol.-% water content, aggregate bands start to emerge.[18] At 30 vol.-% water content,[20] the monomer band is greatly reduced to ~ 10 % of its initial intensity, and both blue- and red-shifted bands at 555 and 761 nm, respectively, were observed. The corresponding

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Scheme 1. Route to chiral squaraine **1** via a two-step, one-pot procedure.
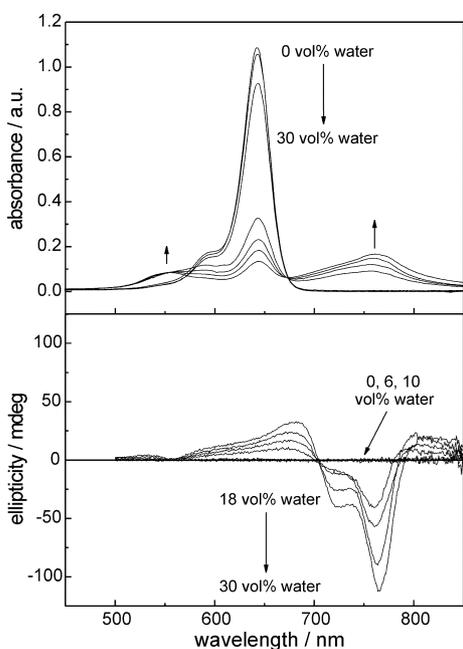
Figure 1. UV-vis absorption (top) and circular dichroism spectra (bottom) of **1** in acetonitrile (3.8×10^{-6} M) containing increasing water content (0, 6, 10, 18, 22, 26, and 30 vol.-%) at 25 °C.

circular dichroism (CD) spectra (Fig. 1, bottom) reveal a strong negative Cotton effect that coincides with the position of the red-shifted absorption band of the aggregate, thereby providing alternative evidence for aggregate formation. The intense observed Cotton effect indicates strong exciton coupling of the individual chromophores within the aggregate and proves their relative chiral orientation.[21] Interestingly, in the wavelength region of the blue-shifted absorption band, only a relatively weak exciton couplet was observed. It should be pointed out that incorporation of enantiopure side chains leads to *direct* observation of the aggregate's overall optical activity. Such excess handedness could not be detected previously in squaraine aggregates based on achiral derivatives unless chiral microenvironments such as enantiopure surfactant micelles[7g] or cyclodextrin hosts[7e] were present.

In contrast to polar analogues of **1**,[16] aggregate solutions were fully equilibrated as indicated by the lack of detectable absorption changes over time. Aggregation studies could only

be carried out at rather low concentrations ($2 \leq c \leq 4$ μM) since below this concentration range no sign of aggregation could be observed, while above this concentration range samples were not equilibrated due to precipitation of the aggregate.[18] Since association processes are naturally dependent on monomer concentration, aggregation of **1** is likely also to occur in nonpolar-organic-solvent mixtures at higher concentrations that are not conducive to optical detection due to the intense absorption of the squaraine chromophore. Indeed, visual inspection of concentrated solutions of **1** in cyclohexane showed a red color that, however, changed to the usual blue color upon dilution.

Similar to solvent composition, temperature also has a marked effect on aggregation of **1** in aqueous solution. This is illustrated by heating a solution of **1** in aqueous acetonitrile containing 30 vol.-% water (Fig. 2). When the temperature is increased from 25 to 60 °C, both aggregate bands almost completely disappear and the monomer band is recovered (Fig. 2, top). The presence of two well-defined isosbestic points at 574
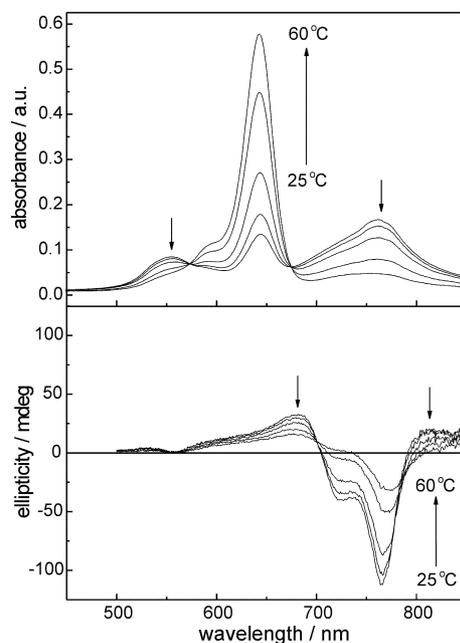
Figure 2. UV-vis absorption (top) and CD spectra (bottom) of **1** in water/acetonitrile mixture (30:70 v/v, 3.8×10^{-6} M) measured at 25, 35, 45, 55, and 60 °C.

and 674 nm, indicates clean conversion of *one* aggregate type to the non-aggregated parent squaraine **1**. This important finding is further supported by the linear nature of the extinction-difference diagrams.[18] In addition, monitoring the disassembly process by CD spectroscopy (Fig. 2, bottom) shows the loss of the sample's ellipticity and the presence of an isodichroic point at 700 nm, thereby independently supporting the presence of only *one* type of aggregate.

In addition to investigating conditions for association,[18] solutions containing aggregates of squaraine **1** were studied by fluorescence spectroscopy to gain further insight into the nature of the aggregate. Solutions containing non-aggregated and aggregated dye **1** showed fluorescence only when the monomeric species was excited (Fig. 3, top). While the spectral shape and Stokes shift ($\Delta\lambda_{max} = 16$ nm) were comparable
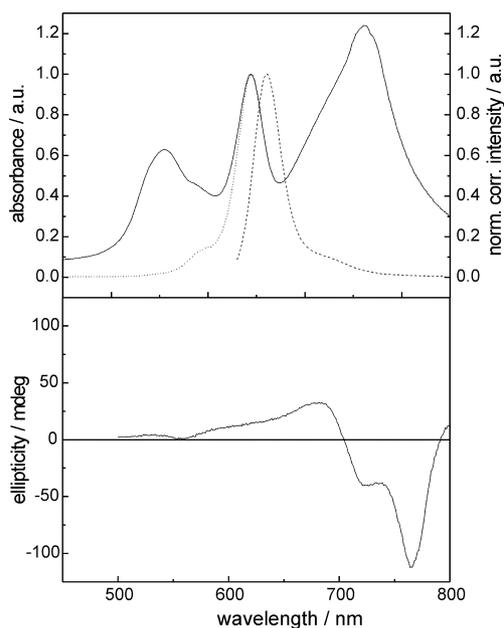

Figure 3. Top: UV-vis absorption (solid) and fluorescence emission (dashed) spectra at $\lambda_{exc} = 620$ nm, and the corresponding excitation spectrum (dotted) at $\lambda_{em} = 660$ nm. Bottom: CD spectrum of **1** in water/acetonitrile mixture (30:70 v/v, 3.8×10^{-6} M) at 25 °C.

to that of the organic solution, the fluorescence quantum yield of the monomeric species under these conditions was significantly decreased ($\Phi_f = 0.31$), implying additional non-radiative decay pathways in aqueous solution. Obtaining an excitation spectrum revealed that emission was arising solely from the monomeric chromophore, as both band position and shape are practically superimposable with the spectra of **1** under non-aggregating conditions.[18] In contrast to the highly emissive non-aggregated dye, chromophore–chromophore interactions within the aggregate seem to enable efficient non-radiative deactivation pathways, leading to complete absence of fluorescence.[7e,g]

The presence of *two* absorption bands arising from only *one* aggregate type, and their large ($\Delta\nu_{agg} = 4880$ cm^{-1}) and sym-

metrical ($\nu_{agg} - \nu_{mon} = 2440$ and -2440 cm^{-1}) splitting, are without literature precedence[22] and imply a unique squaraine aggregate structure. In view of the existing theoretical work on exciton coupling in dye aggregates,[23] the observed large exciton coupling as well as the intrinsic Cotton effect point to the presence of a helical aggregate structure.[24,25]

Aggregation on solid-substrate surfaces[26] was investigated by scanning tunneling microscopy (STM) of self-assembled monolayers (SAMs) at the interface between an organic solution and the basal plane of highly oriented pyrolytic graphite (HOPG).[27] In addition to adsorbate–adsorbate interactions, adsorbate–surface interactions rather than specific solvation constitute the main driving force for aggregation under these conditions. While optical spectroscopy in solution provided valuable insight into the factors that govern squaraine aggregation, STM experiments can reveal additional structural information about self-assembly, at least in two dimensions, due to its inherent high-resolution capability.[27,28] Previously, SAMs of achiral bis(4-dialkylamino-2-hydroxyphenyl)squaraine dyes on HOPG have been studied by STM, and, depending on the length of the alkyl tail, several polymorphs consisting mostly of herringbone- and lamellar-packing patterns could be observed.[29] In comparison, squaraine **1** features a very different molecular geometry due to the presence of two chiral centers serving as attachment points for the *n*-decyl chain, which facilitates SAM formation on HOPG.

After applying solutions of squaraine **1** in trichlorobenzene to a freshly cleaved HOPG surface, rapid formation of periodic structures was observed. Two coexisting yet rather different aggregation patterns, namely honeycomb and moiré structures, could be distinguished (Fig. 4, top).

While the average area of the unit cells in both polymorphs is indistinguishable within experimental error (1.41 ± 0.09 nm^2), the calculated area of the flat, oriented molecule (2.8 ± 0.3 nm^2)[18] is roughly twice as large. The squaraine moiety is attributed to the bright areas corresponding to high tunneling current; it is oriented along the unit-cell diagonal, which almost perfectly matches its length. Knowing the roughly equal spatial requirements of the squaraine and its alkyl chains, we assume a *layered* arrangement in which the alkyl chains form a flat layer in between the HOPG and the squaraine moiety.[18] Such a structure seems more likely than the direct adsorption of the squaraines on HOPG, since the linear alkyl chains have a high affinity for the graphite surface[27] and the squaraine moieties are not aligned with respect to the threefold symmetrical substrate. Although STM is unable to visualize the underlying alkyl chains directly, commensurability with the HOPG substrate should dictate their relative orientation along the graphite axes.[27] The observed differences between the polymorphs most likely originate either from different relative orientations of the extended alkyl chains and/or squaraine–squaraine interactions.

To the best of our knowledge, such *self-templating* pattern formation on HOPG has not been reported in the literature. Although SAMs of amphiphilic molecules have previously successfully been used to facilitate adsorption onto HOPG
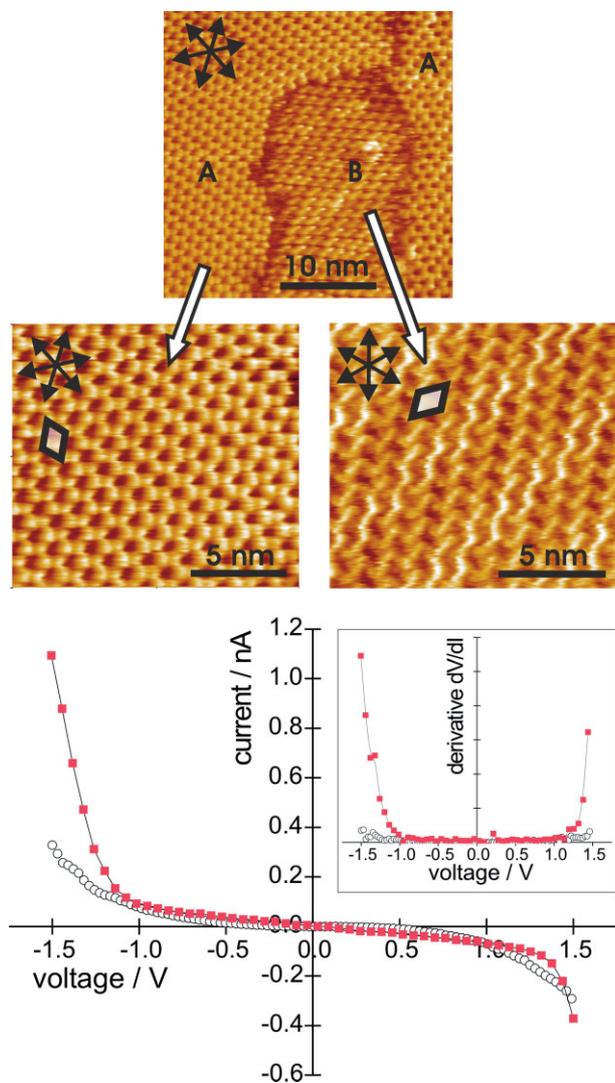
Figure 4. Top: Representative STM current image (raw data) of SAMs of **1** on HOPG prepared from trichlorobenzene (sample bias $U_s = -1.13$ V, tunneling current $I_t = 250$ pA) showing two different domain types (A, B). Center: Higher-magnification images of honeycomb (A, left) and moiré (B, right) patterns including unit cells. Bottom: Current–voltage (*I–V*) characteristics (raw data, averaged over different locations and domains) of honeycomb and moiré patterns (red solid squares) and HOPG [32] (black open circles). The inset shows the first derivative d*V*/d*I*, emphasizing the onset of resonant tunneling through the adsorbate layer.

between the dyes within the 2D crystal, as expected from the similar relative orientation of the chromophores. At larger voltages, the SAMs showed a more pronounced current increase than the HOPG substrate, arising from resonant tunneling through the adsorbate layer.[31] A plot of the first derivative of the current (Fig. 4, bottom inset) provides an estimate for the energetic position of the squaraine highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) levels, with a resulting energy gap (2.2 eV) that is in reasonable agreement with the value obtained from optical spectroscopy (1.9 eV) and with the STS data obtained for a monolayer of squaraine chromophores connected to a gold substrate via a C_3H_6–S tail.[32] A more detailed discussion is difficult, since in the latter case no molecularly resolved structures were reported and, moreover, the symmetry of the *I–V* characteristics varies across the surface. In our case, the slight, highly reproducible asymmetry of the *I–V* characteristics may be attributed to the asymmetry of the energetic position of the squaraine HOMO and LUMO with respect to the Fermi energy of the substrate.[31]

In summary, a novel chiral squaraine dye has been prepared from L-proline via a straightforward synthetic route. Utilizing spectroscopic measurements under various conditions and STM/STS, the aggregation behavior of squaraine **1** has been elucidated. While in aqueous solution aggregation is dominated by the hydrophobic effect, self-assembly on HOPG is governed by alkyl-chain–substrate as well as squaraine–squaraine interactions. Detailed analysis of the STM images has revealed the possibility of an unusual mode of layering on graphite in which the squaraines' alkyl side chains separate the squaraines from the graphite, and at the same time direct self-assembly, thereby giving rise to an unprecedented self-templating effect. The unparalleled aggregation behavior in solution and at the interface is most likely caused by the specific molecular structure as well as by the chirality of the synthesized squaraine dye. These observations should facilitate the future design of related self-assembled materials for (supra)molecular electronics.[33]

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[4] For example: a) C. T. Chen, S. R. Marder, L. T. Cheng, *J. Chem. Soc. Chem. Commun.* **1994**, 259. b) G. J. Ashwell, G. Jefferies, D. G. Hamilton, D. E. Lynch, M. P. S. Roberts, G. S. Bahra, C. R. Brown, *Nature* **1995**, *375*, 385.

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and to induce alignment of various ionic species,[30] assembly on top of the initially formed templating monolayer is not particularly homogeneous. The suggested self-templating effect described herein offers the great advantage that alignment is achieved in a unimolecular, and hence more robust and predictable, fashion.

Local electronic coupling between squaraine moieties within SAMs was probed using scanning tunneling spectroscopy (STS).[31] The measurements of current–voltage (*I–V*) characteristics through honeycomb and moiré structures did not reveal any significant difference between both polymorphs (Fig. 4, bottom). This points to similar electronic interactions

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[9] A selected recent example involving merocyanines is given in: A. Lohr, M. Lysetska, F. Würthner, *Angew. Chem.* **2005**, *117*, 5199; *Angew. Chem. Int. Ed.* **2005**, *44*, 5071.

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[12] C. W. Dirk, W. C. Herndon, F. Cervantes-Lee, H. Selnau, S. Martinez, P. Kalamegham, A. Tan, G. Campos, M. Velez, J. Zyss, I. Ledoux, L. T. Cheng, *J. Am. Chem. Soc.* **1995**, *117*, 2214.

[13] a) K.-Y. Law, F. C. Bailey, *J. Org. Chem.* **1992**, *57*, 3278. b) The recently reported stabilization of squaraines by encapsulation in rotaxanes is not amenable to our design since the shielding also suppresses aggregation: E. Arunkumar, C. C. Forbes, B. C. Noll, B. D. Smith, *J. Am. Chem. Soc.* **2005**, *127*, 3288.

[14] G. J. Ashwell, *J. Mater. Chem.* **1998**, *8*, 373.

[15] M. A. Balbo Block, S. Hecht, *Macromolecules* **2004**, *37*, 4761.

[16] R. S. Stoll, *Diplom Thesis*, Freie Universität Berlin, Germany **2004**.

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[25] Definite structural proof could not be obtained yet, as structure elucidation of aggregates in solution is intrinsically difficult. A more quantitative interpretation of the observed aggregation phenomena in solution has so far been hampered by our inability to determine extinction coefficients of the pure aggregates and their respective aggregation numbers.

[26] Initial work on preparing thin films by drop casting organic solutions of squaraine **1** on quartz glass resulted in films that, after annealing (i.e., heating), displayed spectra that qualitatively reflect the aggregation behavior in aqueous solution displaying blue- and red-shifted aggregate bands. Interestingly, the fine structure of the red-shifted aggregate was much more developed.

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