Lifetime Engineering Hot Paper

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A Macrocyclic Fluorophore Dimer with Flexible Linkers: Bright Excimer Emission with a Long Fluorescence Lifetime

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Abstract: *Bright fluorescent molecules with long fluorescence lifetimes are important for the development of lifetime-based fluorescence imaging techniques. Herein, a molecular design is described for simultaneously attaining long fluorescence lifetime (τ) and high brightness ($\Phi_F \times \varepsilon$) in a system that features macrocyclic dimerization of fluorescent π-conjugated skeletons with flexible linkers. An alkylene-linked macrocyclic dimer of bis(thienylethynyl)anthracene was found to show excimer emission with a long fluorescence lifetime ($\tau \approx 19$ ns) in solution, while maintaining high brightness. A comparison with various relevant derivatives revealed that the macrocyclic structure and the length of the alkylene chains play crucial roles in attaining these properties. In vitro time-gated imaging experiments were conducted as a proof-of-principle for the superiority of this macrocyclic fluorophore relative to the commercial fluorescent dye Alexa Fluor 488.*

Over the last decades, the improved understanding of the relationship between π-conjugated structures and photophysical properties has enabled rational design approaches to "bright" fluorescent molecules. Quantum chemical calculations allow the determination of oscillator strength values, which directly relate to molar absorption coefficients (ε) and thus provide a basis to estimate fluorescence quantum yields (Φ_F).[1] Recently, emissive molecules with long luminescent lifetimes (τ) have attracted particular attention on account of the broad spectrum of their applications, including as security inks and fluorescent labels.[2,3] For application as fluorescent labels, time-gated imaging eliminates autofluorescence, thus offering the possibility to conduct lifetime-based multiplexing assays.[3–5] However, the prediction and engineering of fluorescence lifetimes for organic fluorophores still remains a significant challenge.

For the time-gated imaging of biological samples, a fluorophore should ideally fulfill four requirements: 1) a long fluorescence lifetime, 2) a high fluorescence quantum yield, 3) a large molar absorption coefficient, and 4) an excitation by visible light. To maximize the signal-to-noise ratio, it is crucial to simultaneously attain high brightness ($\Phi_F \times \varepsilon$) and a long fluorescence lifetime ($\tau \approx 10$ ns). However, the Strickler–Berg law dictates that the fluorescence lifetime for a rigid fluorescent molecule with small structural relaxation in the excited state is inversely proportional to the integral of the molar absorption coefficient (Figure 1 a).[6] This is reflected in the fact that most bright molecules with intense absorption and strong fluorescence have relatively short lifetimes.[3] Another critical issue is the excitation wavelength, λ_{ex}: even though some polycyclic-aromatic-hydrocarbon-based organic fluorophores such as pyrene exhibit high τ values, they suffer from the necessity of UV excitation, where phototoxicity poses a serious problem.[3] Even though extensive efforts have been devoted to the development of new fluorophores, and although various long-lived emissive molecules that can be excited by visible light and exhibit high fluorescence quantum yields have been reported, most of these remain limited by the Strickler–Berg law.[7–10] To develop an optimal fluorophore for time-gated imaging, a strategy to circumvent this dilemma is required. Recently, Song and co-workers have, in an elegant approach, employed thermally activated delayed fluorescence to significantly extend the fluorescence lifetime. However, as a consequence of harnessing the triplet excited state, their molecule was susceptible to quenching by molecular oxygen.[11]

Herein, we report the macrocyclic dimerization of a highly fluorescent π skeleton as a promising design principle for emissive molecules that satisfy all the aforementioned requirements. For that purpose, we employed an intrinsically fluorescent π skeleton with high oscillator strength to attain intense absorption and induce excimer formation as a result of the macrocyclization. Although excimer emission generally exhibits a long fluorescence lifetime, it often suffers from a low fluorescence quantum yield as a result of rapid nonradiative decay from the excited state. However, we discovered that the precise tuning of the excimer formation, using a cyclophane-like motif with moderately long alkylene linkers, can provide all of the required properties. Fluorescent

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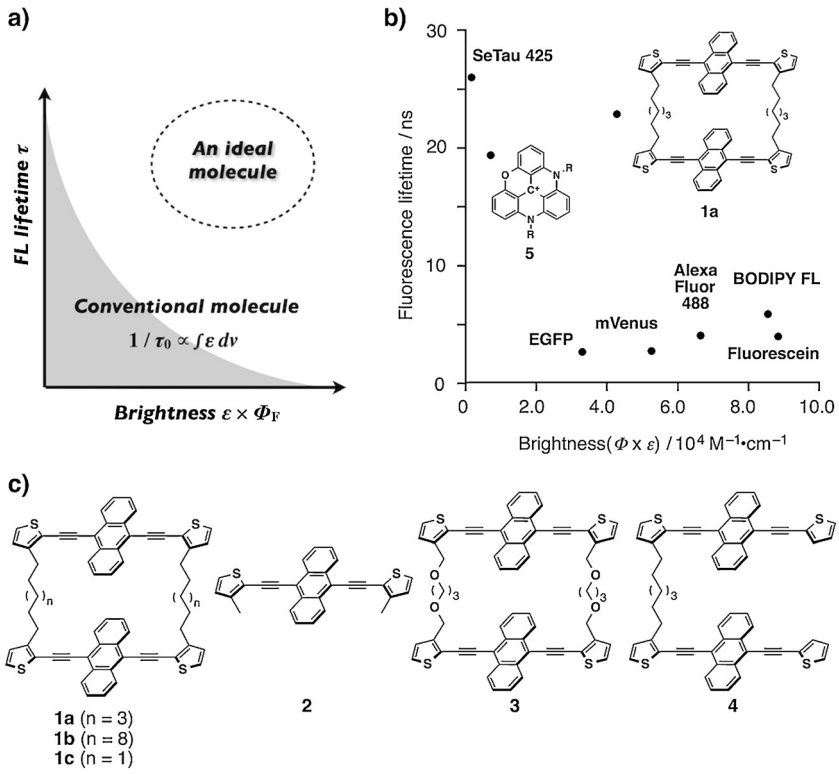

Figure 1. a) A representation of the Strickler–Berg law, wherein τ_0 refers to the natural radiative lifetime ($1/k_r$, where k_r is the radiative rate constant) of the fluorophore. b) A comparison of the fluorescence lifetimes (τ) and brightness values ($\Phi_F \times \varepsilon$) for fluorophores which can be excited by visible light. For **1a**, values in DMF were used. Values for **5**, Alexa Fluor 488, BODIPY FL, and fluorescein, as well as for EGFP and mVenus were adopted from references [9, 17, 18, and 19], respectively. c) Chemical structures of compounds used in this study.

cyclophanes have been extensively investigated in terms of fundamental photophysical properties and molecular recognition, but the majority of these compounds contains rather short linkers to maximize bichromophoric interactions.[12] In contrast, the compounds employed in this study exhibit a dimeric structure of π skeletons in a macrocyclic fashion with flexible alkylene linkers. Previously, we have reported that this structural motif allows the effective tuning of the solid-state morphology.[13] Herein, we disclose that this molecular design also allows the effective engineering of the fluorescence lifetime.

As the fluorescent π skeleton, we chose 9,10-bis(thienylethynyl)anthracene (**2**).[14] Since this anthracene derivative shows intense absorption and emission in the visible region (in CH_2Cl_2: $\lambda_{abs} = 483$ nm, $\varepsilon = 40\,400\ M^{-1}cm^{-1}$, $\lambda_{em} = 503$ nm, $\Phi_F = 0.78$), this skeleton should be a suitable scaffold for obtaining bright and long-lived excimer emission. We therefore prepared a series of macrocyclic dimers of 9,10-bis(thienylethynyl)anthracene (**1a–c**), linked with flexible alkylene tethers of different length (Figure 1c). We envisioned that moderately long alkylene chains, for example, heptylene, should allow conformational flexibility in the ground and excited states. Macrocyclic dimer **3** with more flexible ether linkers and noncyclic dimer **4** were prepared as reference compounds. The synthesis of these compounds is described in detail in the Supporting Information.

Macrocyclic dimer **1a**, containing heptylene linkers, showed a similar absorption spectrum to that of monomer **2**. For **1a**, an absorption maximum and a molar absorption coefficient of $\lambda_{abs} = 491$ nm and $\varepsilon = 64\,700\ M^{-1}cm^{-1}$ were observed, respectively, whereas **2** exhibited the absorption maximum at $\lambda_{abs} = 483$ nm (Figure 2a). The modest bathochromic shift and hypochromism (that is, the molar absorption coefficient of **1a** is less than twice the molar absorption coefficient of **2**) indicate the existence of rather weak ground-state interactions between two chromophores, which is consistent with previous reports on a macrocyclic oligothiophene dimer.[15] In contrast to the absorption, the fluorescence profile of **1a** was very different from that of **2**. In CH_2Cl_2, macrocyclic dimer **1a** showed a dual fluorescence with emission maxima at $\lambda = 515$ nm and 560 nm, whereas monomer **2** only showed structured emission centered at $\lambda = 503$ nm (Figure 2a). The longer-wavelength component most likely arises from the excimer emission (see below). The fluorescence quantum yield of **1a** was high ($\Phi_F = 0.81$), and its brightness in CH_2Cl_2 was $\Phi_F \times \varepsilon =$

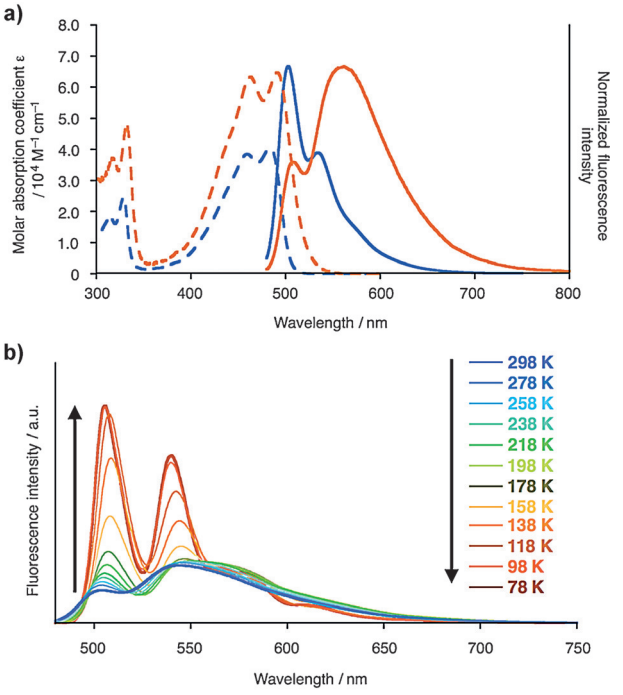

Figure 2. a) UV/Vis absorption (dashed lines) and fluorescence (solid lines) spectra of macrocyclic dimer **1a** (red) and **2** (blue) in CH_2Cl_2 (0.7 μM, 25 °C); b) temperature-dependent fluorescence spectra of **1a** in 2-methyltetrahydrofuran (0.8 μM).

52400 $M^{-1}cm^{-1}$, which is a promising value, especially considering bioimaging applications. Importantly, the time-resolved fluorescence for **1a** in CH_2Cl_2 is best described by a biexponential decay, and the observed longer lifetime component ($\tau = 18.9$ ns; see Table S1 in the Supporting Information) satisfies the requirement for time-gated imaging.

The dual emission property of **1a** was further investigated by low-temperature fluorescence measurements. With decreasing temperature, the structured emission band resembling monomer **2** was restored concomitant with a loss of the structureless emission in the longer-wavelength region (Figure 2b). In addition, time-resolved fluorescence measurements showed that the shorter lifetime component can be attributed to the emission in the shorter wavelength region (Figure S20). In light of the fact that the shorter lifetime component (2.9 ns) was comparable to that of monomer **2** (2.7 ns), the faster decay component should correspond to the monomeric emission. The fluorescence spectrum of **1a** in CH_2Cl_2 ([**1a**] = 700 nM) was virtually identical to that of a lower concentration ([**1a**] = 35 nM), excluding the possibility of an intermolecular excimer (Figure S12). In conclusion, we ascribed the longer wavelength emission of **1a** to intramolecular excimer emission.

While investigating the photophysical properties of **1a** in detail, we found that the monomer/excimer ratio of **1a** is highly dependent on the solvent polarity (Figure S1; Table S1).[16] For instance, in nonpolar solvents such as toluene, the monomer emission is predominantly observed together with a marked decrease of the longer lifetime component. Conversely, in polar solvents such as DMF, the excimer emission dominates. Notably, despite the marked increase in the excimer ratio in DMF, the fluorescence quantum yield remained high ($\Phi_F = 0.71$, brightness = 42000 $M^{-1}cm^{-1}$). This result suggests that intense excimer emission can be expected from **1a** even in biological media.

A comparison of **1a** with known fluorophores that can be excited by visible light is summarized in Figure 1b.[9,17–19] Although many of the known bright fluorophores surpass **1a** in terms of brightness, their fluorescence lifetimes are too short to eliminate autofluorescence by time-gated imaging, as the lifetime of autofluorescence is comparable with these fluorophores.[3,9c] In contrast, the fluorescence lifetime of **1a** is long enough for background elimination. Moreover, the brightness of **1a** is considerably higher in comparison with the previously reported long-lived emissive molecule **5**[9a] and commercially available SeTau 425 (Figure 1b). These results clearly demonstrate the superiority of **1a** as a bright fluorophore with a long fluorescence lifetime for time-resolved imaging applications.

To elucidate the role of the linkers, the fluorescence properties of macrocyclic dimers with varying linker length in CH_2Cl_2 were compared (Table S1). As expected, dodecylene-linked dimer **1b** exhibited a distinct decrease in the excimer/monomer ratio (Figure S3). In contrast to our expectation that the shorter linker should increase the excimer/monomer ratio, the fluorescence spectrum of pentylene-linked dimer **1c** was similar to that of **1a** (Figure S5). Interestingly, the fluorescence quantum yield of **1c** ($\Phi_F = 0.66$) was lower than that of **1a** ($\Phi_F = 0.81$). In order to obtain optimal photophysical properties, the linker should thus neither be too long, nor too short.

The impact of the nature of the linker was further investigated by comparison with derivative **3**, which contains more flexible 2,6-dioxaheptylene linkers, and with noncyclic, singly bridged dimer **4**. In accordance with previous reports,[20] a comparison of **3** and **1a** showed that the more flexible ether linker largely increases the excimer ratio (Figure S8), albeit with lower brightness ($\varepsilon = 54700$ $M^{-1}cm^{-1}$, $\Phi_F = 0.61$, brightness = 33400 $M^{-1}cm^{-1}$). In contrast, a comparison between noncyclic dimer **4** and macrocyclic dimer **1a** demonstrated the significance of the macrocyclic structure on the fluorescence properties. Although the length of the alkylene linker is identical, noncyclic dimer **4** showed a decreased excimer/monomer ratio. Interestingly, the fluorescence spectra of **4** were rather insensitive towards solvent polarity (Table S1; Figure S10). This behavior is in stark contrast to macrocyclic dimer **1a**, implying that the macrocyclic scaffold plays an important role in favoring the excimer formation in polar solvents. The macrocyclic motif is therefore crucial for maximizing the excimer emission in polar media, which should be important for future biological applications.

To gain deeper insight into the nature of the excited state of the macrocyclic dimers, we performed theoretical calculations for both the ground state and the first excited state. As a result of the presence of flexible alkylene chains, the presence of various local minima should be expected, which would hamper a density functional theory (DFT)-assisted structural optimization. Therefore, we resorted to molecular dynamics (MD) simulations using DFTB2,[21] which is an approximation method derived from DFT, as well as to a modified, purely classical MM3 force field approach.[22–24] Starting from the previously obtained crystal structure,[13b] the MD simulations at two different levels of theory yielded two distinct structures, which we refer to in the following as "cross" (MM3) and "parallel" (DFTB2) stacks (Figure 3a). However, the energy differences between the two stacked conformers, obtained by single-point calculations, were dependent on the calculation methods (Table S3), and therefore, we conclude at this stage that possibly both stack(s) are responsible for the observed excimer formation. Accordingly, we investigated both geometries as potential candidates for the excimer emission, rather than focusing on either of the conformations.

The calculation of the excited state at the CAM-B3LYP-(D3)/6-31G(d) level of theory implied that both stacks could be responsible for the observed excimer emission (Table S3 and Figure S22). Compared to the monomer, intramolecular stacking in a cross or parallel fashion in **1a** resulted in bathochromic shifts of the maximum emission wavelength by 100 nm and 180 nm, respectively. Conversely, single-point calculation of a nonstacked geometry, determined by X-ray diffraction analysis,[13b] furnished an emission maximum similar to that of the monomer. Detailed structural analyses demonstrated that the conformations of the alkylene linkers do not significantly change between the ground and the excited state in both cross and parallel stacks (Table S5). In addition, the dihedral angles between the terminal thiophene

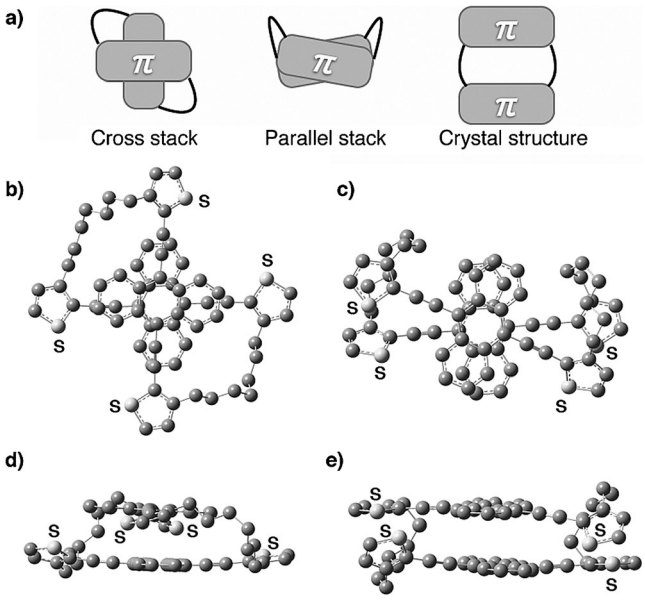

Figure 3. a) Representations of the intramolecular alignment of two chromophores in **1a**, obtained from either MD simulations or the crystal structure, where rectangles and lines represent the π-conjugated skeleton and alkylene chains, respectively. b–e) Optimized structures of **1a** in the ground state using MD simulations based on b, c) MM3 (cross stack) or d, e) DFTB2 (parallel stack). Top and side views of cross-stacked (b, d) and parallel-stacked (c, e) **1a**. Hydrogen atoms are omitted for clarity.

rings and the central anthracene plane showed relatively small differences, thus implying that co-planarization of the π skeleton is not responsible for the observed red-shifted emission bands (Table S6). The most striking structural change between the ground and excited state is the intramolecular distance between the two anthracene moieties. For both stacks, the interatomic distances between the two central benzene rings of the anthracene moieties were shortened by about 0.08–0.10 Å. The average interatomic distances, that is, 3.336 Å for cross stacks and 3.261 Å for parallel stacks, were below the sum of the van der Waals radii (Table S4). Therefore, the observed red shift for both stacks should most likely arise from the excimer-like interaction, where stabilization by overlap of molecular orbitals overcomes electrostatic repulsion in the excited state.

Although we are currently still unable to offer a definite explanation for the role of the alkylene linkers in causing such an intense excimer emission, we believe them to favor the excimer formation by way of an entropic factor. Unlike pyrene, which shows excimer emission at a relatively low concentration, anthracene requires highly concentrated solutions in order to induce the excimer emission. This different behavior is probably due to the difference in the excited-state lifetime.[25] The same is probably also true for the present case, where a concentrated solution of monomer **2** ($[\mathbf{2}] \leq 5$ mM) did not show any significant excimer emission (Figure S13). The macrocyclic structure with the appropriate alkylene linkers is therefore most likely responsible for decreasing possible conformations and favoring preorganization in the ground state, thus populating stacked conformations that readily undergo excimer formation in the excited state.[26]

Finally, to demonstrate the potential utility of **1a** for lifetime-based fluorescence imaging, we examined the in vitro time-gated separation of **1a** and Alexa Fluor 488 (hereafter referred to as AL488; $\tau = 4.1$ ns), which is a representative commercial fluorescent dye for bioimaging. For that purpose, we prepared a microfluidic device, containing reservoirs for a DMF solution of **1a** and an aqueous solution of AL488. Time-gated fluorescence images of each reservoir were collected independently, before fluorescence intensities of **1a** and AL488 were compared (Figure 4). Notably, after

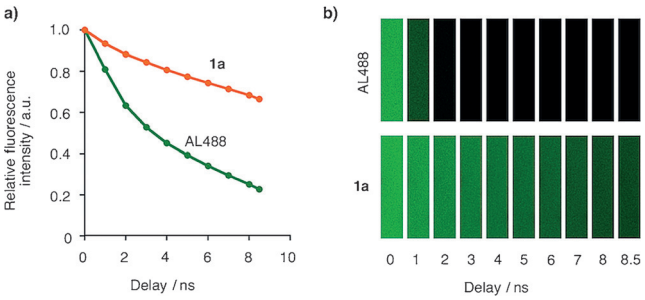

Figure 4. a) Relative fluorescence intensity of **1a** (in DMF; orange) and AL488 (in pH 7.4 HEPES buffer; green) plotted against the delay of time-gating (gating time = 3.5 ns; for example, for a delay of 2 ns, fluorescence signals between 2.0–5.5 ns after the excitation were integrated). b) Confocal micrographs of each time-gated image. Excitation laser $\lambda_{ex} = 488$ nm.

a delay of 7 ns (gating time = 3.5 ns), the fluorescence intensity of AL488 decreased to 29% of the initial value, whereas the intensity of **1a** remained as high as 71%. It is important to note that although the initial fluorescence intensity of AL488 is about 1.6 times higher than that of **1a**, the fluorescence intensity of **1a** is, after a delay of 8.5 ns, about 1.9 higher than that of AL488 (Table S2). This result implies potential for **1a** in time-gated imaging applications, even when autofluorescence is significantly stronger than the desired fluorescence.

In summary, we have developed a bright and long-lived fluorescent molecule by confining two π skeletons into a macrocycle by using moderately long alkylene linkers. The obtained macrocyclic dimer showed intense absorption and emission, together with a significantly increased fluorescence lifetime in comparison with conventionally used fluorescent probes. An examination of the structure–property relationship between the linker length and the emission revealed that the length of the linker indeed has a crucial impact on the emission properties. To show the utility of **1a** for lifetime-based multiplexing and autofluorescence elimination, we successfully demonstrated the superior fluorescence performance of **1a** relative to AL488. The development of water-soluble analogues of **1a** that are suitable for intracellular and in vivo imaging is currently in progress in our laboratories.

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[1] J. R. Lacowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed., Springer, Berlin, **2006**.
[2] a) Y. Lu, J. Zhao, R. Zhang, Y. Liu, D. Liu, E. M. Goldys, X. Yang, P. Xi, A. Sunna, J. Lu, Y. Shi, R. C. Leif, Y. Huo, J. Shen, J. A. Piper, J. P. Robinson, D. Jin, *Nat. Photonics* **2014**, *8*, 32; b) Z. An, C. Zheng, Y. Tao, R. Chen, H. Shi, T. Chen, Z. Wang, H. Li, R. Deng, X. Liu, W. Huang, *Nat. Mater.* **2015**, *14*, 685.
[3] M. Y. Berezin, S. Achilefu, *Chem. Rev.* **2010**, *110*, 2641.
[4] a) L. Gu, D. J. Hall, Z. T. Qin, E. Anglin, J. Joo, D. J. Mooney, S. B. Howell, M. J. Sailor, *Nat. Commun.* **2013**, *4*, 2326; b) J. Joo, X. Liu, V. R. Kotamraju, E. Ruoslahti, Y. Nam, M. J. Sailor, *ACS Nano* **2015**, *9*, 6233.
[5] a) L. Zhang, X. Zheng, W. Deng, Y. Lu, S. Lechevallier, Z. Ye, E. M. Goldys, J. M. Dawes, J. A. Piper, J. Yuan, M. Vereist, D. Jin, *Sci. Rep.* **2014**, *4*, 6597; b) K. Hoffmann, T. Behnke, D. Drescher, J. Kneipp, U. Resch-Genger, *ACS Nano* **2013**, *7*, 6674.
[6] S. J. Strickler, R. A. Berg, *J. Chem. Phys.* **1962**, *37*, 814.
[7] a) T. Hirose, Y. Tsunoi, K. Matsuda, *Chem. Eur. J.* **2015**, *21*, 1637; b) S. Maniam, R. P. Cox, S. J. Langford, T. D. M. Bell, *Chem. Eur. J.* **2015**, *21*, 4133; c) P. Audebert, F. Miomandre, G. Clavier, M.-C. Verniéres, S. Badré, R. Méallet-Renault, *Chem. Eur. J.* **2005**, *11*, 5667; d) S. Kato, T. Furuya, M. Nitani, N. Hasebe, Y. Ie, Y. Aso, T. Yoshihara, S. Tobita, Y. Nakamura, *Chem. Eur. J.* **2015**, *21*, 3115; e) J. A. Smith, R. M. West, M. Allen, *J. Fluoresc.* **2004**, *14*, 151; f) R. Ziessel, L. Bonardi, P. Retailleau, G. Ulrich, *J. Org. Chem.* **2006**, *71*, 3093.
[8] a) A. Fukazawa, M. Hara, T. Okamoto, E.-C. Son, C. Xu, K. Tamao, S. Yamaguchi, *Org. Lett.* **2008**, *10*, 913; b) A. Fukazawa, E. Yamaguchi, E. Ito, H. Yamada, J. Wang, S. Irle, S. Yamaguchi, *Organometallics* **2011**, *30*, 3870.
[9] a) E. Thyrhaug, T. J. Sørensen, I. Gryczynski, Z. Gryczynski, B. W. Laursen, *J. Phys. Chem. A* **2013**, *117*, 2160; b) B. P. Maliwal, R. Fudala, S. Raut, R. Kokate, T. J. Sørensen, B. W. Laursen, Z. Gryczynski, I. Gryczynski, *PLOS One* **2013**, *8*, e63043; c) R. M. Rich, D. L. Stankowska, B. P. Maliwal, T. J. Sørensen, B. W. Laursen, R. R. Krishnamoorthy, Z. Gryczynski, J. Borejdo, I. Gryczynski, R. Fudala, *Anal. Bioanal. Chem.* **2013**, *405*, 2065.
[10] A. Shivalingam, M. A. Izquierdo, A. L. Marios, A. Vysniaukus, K. Suhling, M. K. Kuimova, R. Vilar, *Nat. Commun.* **2015**, *6*, 8178.
[11] X. Xiong, F. Song, J. Wang, Y. Zhang, Y. Xue, L. Sun, N. Jiang, P. Gao, L. Tian, X. Peng, *J. Am. Chem. Soc.* **2014**, *136*, 9590.
[12] a) D. Ramaiah, P. P. Neelakandan, A. K. Nair, R. R. Avirah, *Chem. Soc. Rev.* **2010**, *39*, 4158; b) J. Ferguson, *Chem. Rev.* **1986**, *86*, 957.
[13] a) S. Saito, K. Nakakura, S. Yamaguchi, *Angew. Chem. Int. Ed.* **2012**, *51*, 714; *Angew. Chem.* **2012**, *124*, 738; b) C.-M. Chou, S. Nobusue, S. Saito, D. Inoue, D. Hashizume, S. Yamaguchi, *Chem. Sci.* **2015**, *6*, 2354.
[14] a) C. Wang, Y. Liu, Z. Ji, E. Wang, R. Li, H. Jiang, Q. Tang, H. Li, W. Hu, *Chem. Mater.* **2009**, *21*, 2840; b) Very recently, fluorescent cyclophanes based on bis(phenylethynyl)anthracene have been reported. Y. Sagara, Y. C. Simon, N. Tamaoki, C. Weder, *Chem. Commun.* DOI: 10.1039/C6CC01614F.
[15] T. Sakai, T. Satou, T. Kaikawa, K. Takimiya, T. Otsubo, Y. Aso, *J. Am. Chem. Soc.* **2005**, *127*, 8082.
[16] A similar solvent dependence has previously been observed for [2.2](1,3)pyrenophane. For details, see: T. Hayashi, N. Mataga, T. Umemoto, Y. Sakata, S. Misumi, *J. Phys. Chem.* **1977**, *81*, 424. Although we cannot exclude the possibility of such a solvent-induced polarization, the solvent effect observed for **1a** cannot be directly linked with pyrenophane, as MD simulations predict different ground-state conformations for both compounds.
[17] L. D. Lavis, R. T. Raines, *ACS Chem. Biol.* **2008**, *3*, 142.
[18] O. Shimomura, *Bioluminescence: Chemical Principles and Methods*, Revised ed., World Scientific, Singapore, **2012**.
[19] J. Iain, M. T. Z. Spence, *Molecular Probes Handbook: A Guide to Fluorescent Probes and Labeling Technologies*, 11th ed., Life Technologies, California, **2010**.
[20] W. Rettig, B. Paeplow, H. Herbst, K. Müllen, J.-P. Desvergne, H. Bouas-Laurent, *New J. Chem.* **1999**, *23*, 453.
[21] M. Elstner, D. Porezag, G. Jungnickel, J. Elsner, M. Haugk, Th. Frauenheim, S. Suhai, G. Seifert, *Phys. Rev. B* **1998**, *58*, 7260.
[22] N. L. Allinger, Y. H. Yuh, J. H. Lii, *J. Am. Chem. Soc.* **1989**, *111*, 8551.
[23] J. H. Lii, N. L. Allinger, *J. Am. Chem. Soc.* **1989**, *111*, 8566.
[24] J. H. Lii, N. L. Allinger, *J. Am. Chem. Soc.* **1989**, *111*, 8576.
[25] J. K. McVey, D. M. Shold, N. C. Yang, *J. Chem. Phys.* **1976**, *65*, 3375.
[26] The excitation spectra for the excimer emission showed small bathochromic shifts compared to the monomer emission, thus implying that the geometric difference in the ground state probably plays an important role. For details, see the Supporting Information.

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