A highly efficient sensor molecule emitting in the near infrared (NIR): 3,5-distyryl-8-(*p*-dimethylaminophenyl)-difluoroboradiaza-*s*-indacene

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The spectroscopic properties and the photophysical behaviour of difluoroboradiaza-*s*-indacene **1**, especially designed for the near infrared (NIR) spectral region and equipped with a *p*-dimethylaminophenyl group at the *meso*-position, were studied by steady-state and time-resolved optical spectroscopy. Solvent-dependent measurements revealed that for **1**, excited state deactivation is governed by population of a non-emissive charge transfer excited state (^{1}CT) as the solvent polarity increases, whereas reference compound **2** shows strong fluorescence from a locally excited state (^{1}LE) in all the solvents employed. Accordingly, protonation of **1** completely suppresses the quenching excited state charge transfer process and leads to strong enhancement of fluorescence in the NIR, distinguishing **1** as a very sensitive fluorescent sensor molecule for pH or solvent acidity in this favourable wavelength region.

1 Introduction

Difluoroboradiaza-*s*-indacenes (boron–dipyrromethene dyes, BDPs) are a class of highly rigidized, polymethine-like fluorescent dyes that have found widespread application as laser dyes, as well as in biological research, *e.g.* for protein or DNA labelling.[1] They present many advantageous photonic properties, such as high extinction coefficients, high fluorescence quantum yields and good photostability, and can be excited at wavelengths ⩾500 nm.[2] Lately, the functionalization of BDP dyes has received much attention because of the need for fluorescent labels emitting in different wavelength regions and especially in the near infrared (NIR). Wavelength tuning of the BDP chromophore can be accomplished by attaching auxochromic substituents to the 3,5-positions.[3] Using aromatic groups[3a–c] or heterocyclic moieties,[3d,e] it is possible to shift both absorption and emission bands by 100–200 nm to the red. However, up to now, none of these advanced BDP dyes has been equipped with a third functional group† which, in a certain environment, can drastically influence the spectroscopic properties of the chromophore by activation of an intramolecular photophysical process, such as, for instance, charge (CT) or electron (ET) transfer. Obviously, such an approach would be highly suitable for photonic sensing or switching applications in the NIR.[4,5]

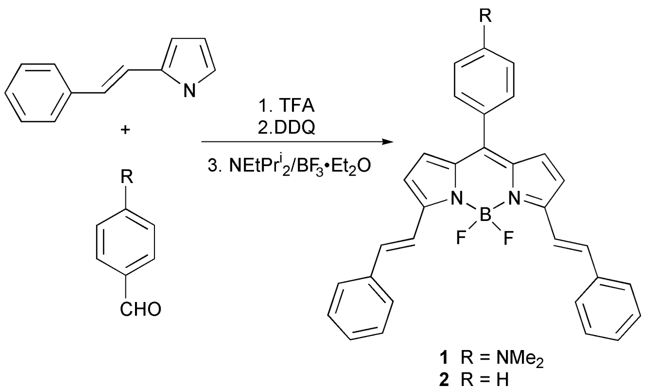
As an extension of our work on charge transfer-active boron–dipyrromethene dyes,[6] we have embarked on the development of NIR-emitting BDP fluorosensors containing electron-donating amino groups, thus enabling us to detect with a high degree of sensitivity free (solvated) protons in this advantageous wavelength region. Here, we report the synthesis (Scheme 1), spectroscopic properties, photophysical mechanisms and fluorescence switching behavior of the symmetrically styryl-substituted BDP dye **1**.

2 Results and discussion

2.1 Spectroscopic properties and photophysical behaviour in non-acidic media

The absorption and fluorescence properties of the reference compound **2** are essentially solvent independent. The absorption spectrum (Fig. 1b) presents the typical BDP features,[2,6] showing the narrow and intense ($\varepsilon = 104\,000$ M^{-1} cm^{-1} in acetonitrile) structured $S_1 \leftarrow S_0$ transition with a maximum at 630 nm which is red-shifted by 130 nm as compared to conventional BDP dyes. The fluorescence spectrum, a mirror-image of the absorption spectrum, is only slightly Stokes-shifted, high fluorescence quantum yields are found and *trans*–*cis* isomerisation is not observed.

1 shows an additional, broad and structureless band in the



1 R = NMe$_2$
2 R = H

Scheme 1 Synthetic scheme for the dyes investigated.

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† A third group, because all BDP dyes of this type synthesized so far are symmetrically substituted at both the 3- and 5-positions.

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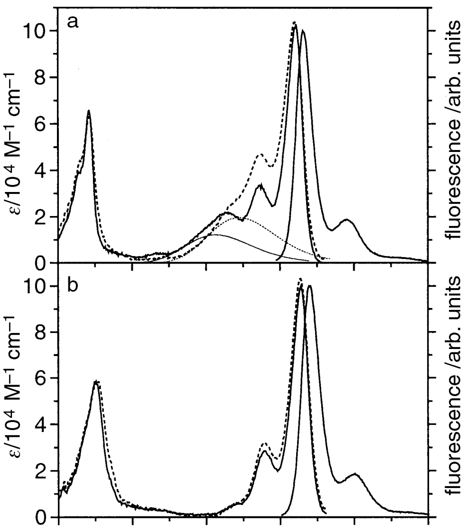

Fig. 1 Absorption and fluorescence spectra of **1** (a) and **2** (b) in acetonitrile (···) and diethyl ether (——) at 298 K (λ_{exc} 595 nm). The emission spectra in acetonitrile, identical in shape, are omitted for better clarity. The thin lines in the top graph represent the CT absorption bands as derived from a decomposition procedure given in the Experimental.

absorption spectrum (Fig. 1a and data in Table 1) which largely overlaps with the low energy $S_1 \leftarrow S_0$ transition located on the BDP chromophore. In accordance with studies on other biaryls[7] which do not carry sterically demanding substituents in direct neighborhood of the interannular bond,‡ this band can be attributed to a charge transfer transition (^{1}CT ← S_0) involving the amino group and the BDP chromophore. In contrast to the $S_1 \leftarrow S_0$ and $S_2 \leftarrow S_0$ (at 350 nm) transitions leading to π–π* locally excited (LE) states of the BDP chromophore, this band shows a strong positive solvatochromism commensurate with the stabilization of the CT state with increasing solvent polarity.

The emission behavior of **1** is strongly solvent-dependent (Table 1). In non-polar solvents the emission is strong and the shape of the fluorescence band is similar to that of **2**, *i.e.* emission only occurs from the BDP LE state. In more polar solvents the characteristic BDP fluorescence is drastically quenched (by a factor of $\leqslant 2 \times 10^4$) with both fluorescence quantum yield and lifetime decreasing at the same time (Table 1). No red-shifted, broad and solvent polarity-dependent band originating from CT fluorescence is found in the emission spectrum. Whereas the radiative rate constant, with similar values to **2**, does not change, the quenching reaction is accompanied by a drastic increase in the non-radiative rate constant. Obviously, the stabilization of the CT state opens up a relaxation pathway for the LE state that proceeds entirely *via* non-radiative processes.

2.2 Proton-induced effects

Upon protonation, the dimethylamino group changes from an electron donor to an acceptor in such a way that any CT interaction is completely suppressed. In the absorption spectrum, the CT band disappears and the LE absorption is shifted to longer wavelengths (Table 1, Fig. 2). The latter can be explained by the fact that, whereas the HOMO of the BDP chromophore has a node at the bridgehead carbon, a large coefficient is found for the LUMO at that position and conversion of the 8-donor to an 8-acceptor lowers the transition energy.§ Consequently, binding of a proton also inhibits the fast excited state intramolecular quenching reaction, resulting in spectrosopic features which are very similar to those of **2** in the respective solvents (Table 1). Therefore, **1**, which is essentially non-fluorescent in a highly polar environment, becomes strongly fluorescent in the presence of protons.

The pK_a of **1**, determined as 2.32, lies in the usual range of values reported for other 8-dimethylaminophenyl-substituted BDP[8] or related donor–acceptor-substituted biaryl dyes, such as *p*-anthracen-9-yldimethylaminobenzene (pK_a = 2.5 in ethanol–water 4 : 1)[9] or 4-dimethylamino-4′-cyano-biphenyl (pK_a = 2.35 in ethanol–water 1 : 1)[5].

Comparing the fluorescence intensity and lifetime data of **1** and **1**–H$^+$ in Table 1, this styryl-substituted BDP dye is dis-

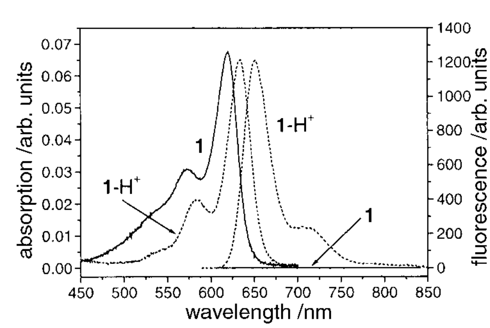

Fig. 2 Absorption and emission spectra of **1** (——) and **1**–H$^+$ (···) in acetonitrile at 298 K ($c_1 = 6.5 \times 10^{-7}$ M, $c_{H+} = 1 \times 10^{-4}$ M).

Table 1 Spectroscopic data for **1**, **2** and **1**–H$^+$ in different solvents at 298 K[a]

	Solvent[b]	$\lambda_{abs}{}^c$/nm	$\lambda_{abs}^{CT}{}^c$/nm	λ_{em}/nm	Φ_f	τ_f/ps	$k_f{}^d/10^8$ s^{-1}	$k_{nr}{}^d/10^8$ s^{-1}
1	MeCN	620	545	636	4×10^{-4}	<3	>1.3	>3330
1	THF	626	522	637	0.017	42	1.4	80
1	Et$_2$O	622	513	631	0.75	4340	1.7	0.6
1	Hex	623	510	631	0.92	4870	1.9	0.2
2	MeCN	628	—	642	0.84	4860	1.7	0.3
2	THF	633	—	646	0.83	4560	1.8	0.4
2	Et$_2$O	628	—	641	0.81	4830	1.7	0.4
2	Hex	630	—	639	0.70	4840	1.4	0.6
1–H$^+$	MeCN	634	—	652	0.75	4820	1.6	0.5
1–H$^+$	THF	637	—	656	0.67	4390	1.5	0.7

[a] Experimental conditions: $c_{dye} = 1 \times 10^{-6}$ M, λ_{exc} = 595 nm for steady-state, 595 and 327 nm for time-resolved fluorescence measurements, see Experimental. [b] Hex = *n*-hexane. [c] Global maximum and maximum of the CT absorption band, for the fitting procedure, see Experimental. [d] $k_f = \Phi_f/\tau_f$, $k_{nr} = (1 - \Phi_f)/\tau_f$.

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‡ Ground state geometry optimization of **1** by the AM1 method (AMPAC 5.0, Semichem, Inc., 1994) revealed a twist angle between the anilino and the BDP fragment of *ca.* 50°, comparable to twist angles found for related biaryls.[7]

§ Calculations on the electronic structure of the BDP core were performed using the AM1 method with VAMP 6.5. (G. Rauhut, A. Alex, J. Chandrasekhar, T. Steinke, W. Sauer, B. Beck, M. Hutter, P. Gedeck and T. Clark, Erlangen, 1996).

tinguished as a very powerful NIR fluorosensor showing enhancement factors of *ca.* 2000. Analytical applications would include, for instance the detection of traces of free (solvated) protons in polar solvents or the monitoring of pH by employing, *e.g.* time-resolved fluorometry with pulsed laser diodes in a simple single excitation/single emission wavelength operation mode.

3 Conclusion

The studies presented reveal that the unique features of the difluoroboradiaza-*s*-indacene chromophore, such as high molar extinction coefficients and fluorescence quantum yields, are maintained when shifting the absorption and emission to the red by elongation of the conjugated electron system, *i.e.* by substitution at the 3- and the 5-positions. Additionally, introducing a strongly electron donating aminophenyl group to the *meso*-position induces a quenching intramolecular charge transfer process in the excited state. With this advanced chromophoric design, a very efficient proton-triggered "switching on" of the fluorescence can be achieved in the NIR.

4 Experimental

4.1 Materials

All the solvents employed for the spectroscopic measurements were of spectroscopic grade and purchased from Aldrich. Perchloric acid (70%, Suprapur, Merck)/hydrochloric acid (30%, Suprapur, Merck) were used for the protonation experiments in organic/mixed aqueous solvents. Doubly distilled water (pH 6.39) was provided by the Laboratory for Trace Elemental Analysis, BAM, Berlin.

4.2 Synthesis

1: 200 mg (1.2 mmol) 2-styrylpyrrole[10] and 320 mg (2.4 mmol, 4-fold excess) 4-dimethylaminobenzaldehyde were stirred in 50 ml CH_2Cl_2 containing two drops of trifluoroacetic acid for 5 h under a N_2-atmosphere. 260 mg (1.2 mmol) DDQ was added and stirring was continued for 15 min, followed by the addition of 3 ml *N*-ethyldiisopropylamine and 3 ml $BF_3 \cdot Et_2O$. After stirring for another 30 min, the reaction mixture was washed with water, dried and the solvent evaporated. Column chromatography of the residue (SiO_2, CH_2Cl_2–petroleum ether 1 : 1, $R_f = 0.8$, blue band) followed by recrystallisation from $CHCl_3$–hexane yields **1** as a purple microcrystalline powder.

Yield: 46 mg (0.09 mmol, 15%); mp: 335–338 °C; IR (KBr: $\tilde{v}$ (cm^{-1}): 1604, 1528 (C=N), 1123 (B–F); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.09 (s, 6 H), 6.80 (d, 2 H, $J = 9.1$), 6.95 (s, 4 H), 7.25–7.45 (m, 8 H), 7.48 (d, 2 H, $J = 9.1$), 7.66 (m, 4 H), 7.81 (d, 2 H, $J = 16.5$ Hz); HRMS (EI, 70 eV) *m*/*z*: calc. for $C_{33}H_{28}N_3BF_2$ 515.2344, found 515.2344 ($\Delta = 0.0$ ppm).

2: 338 mg (2 mmol) 2-styrylpyrrole[10] and 106 mg (1 mmol) benzaldehyde were stirred in 50 ml CH_2Cl_2 containing two drops of trifluoroacetic acid for 5 h under a N_2-atmosphere. 227 mg (1 mmol) DDQ was added and stirring was continued for 15 min, followed by the addition of 3 ml *N*-ethyldiisopropylamine and 3 ml $BF_3 \cdot Et_2O$. After stirring for another 30 min, the reaction mixture was washed with water, dried and the solvent evaporated. Column chromatography of the residue (SiO_2, CH_2Cl_2–petroleum ether 1 : 1, $R_f = 0.8$, red fluorescing band) followed by recrystallisation from $CHCl_3$–hexane yields **2** as purple needles.

Yield: 40 mg (0.08 mmol, 8%); mp: 273–276 °C; IR (KBr: $\tilde{v}$ (cm^{-1}) 1544 (C=N), 1131 (B–F); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.82 (d, 2 H, $J = 4.5$), 6.94 (d, 2 H, $J = 4.5$), 7.29–7.55 (m, 15H), 7.35 (d, 2 H, $J = 16.5$), 7.80 (d, 2 H, $J = 16.5$ Hz); HRMS (EI, 70 eV) *m*/*z*: calc. for $C_{31}H_{23}N_2BF_2$ 472.1922, found 472.1921 ($\Delta = 0.2$ ppm).

4.3 Steady-state absorption and fluorescence spectroscopy

UV/Vis-spectra were recorded on a Bruins Instruments Omega 10 absorption spectrometer and steady-state emission spectra on a Spectronics Instruments 8100 spectrofluorometer. The fluorescence experiments, the determination of the relative fluorescence quantum yields (Φ_f) and the correction of the fluorescence spectra were carried out as described in ref. 6*c*, *d*. Cresyl Violet in methanol ($\Phi_f = 0.54 \pm 0.03$)[11] and Rhodamine 101 in ethanol ($\Phi_f = 1.00 \pm 0.02$)[12] were used as fluorescence standards. The uncertainties of the measurement were determined to ±5% (for $\Phi_f > 0.5$), ±20% (for **1** in THF) and ±30% (for **1** in acetonitrile), respectively.

4.4 Time-resolved fluorescence spectroscopy

Fluorescence lifetimes (τ_f) were measured employing a unique laser impulse fluorometer with ps time resolution. The sample was excited with a synchronously pumped dye laser (Spectra Physics Model 3500 and Model 2760; laser dye: Rhodamine 6G from Lambda Physik) pumped by the 1.2 W single-line output (514 nm) of a mode-locked argon ion laser (Spectra Physics Model 2040E-15S and Model 451, FWHM 100 ps) providing pulses with 1.6 ps duration at a repetition rate of 82 MHz. For excitation in the $S_2 \leftarrow S_0$ band, the third harmonic output [sum frequency of the ground wave and the second harmonic (LBO crystal), generated in a BBO crystal] of a regenerative mode-locked argon ion laser-pumped Ti : sapphire laser was used.[13] For further details on the experimental setup, the recording of the fluorescence decay profiles and data analysis, see ref. 6*c*, *d*. The experimental accuracy was determined to ±3 ps and temporal calibration of the experimental setup was checked with Pinacyanol in ethanol ($\tau_f = 13$ ps ± 1 ps),[14] Rose Bengal in methanol ($\tau_f =$ 0.50 ns ± 0.02 ns)[12] and Cresyl Violet in methanol ($\tau_f = 3.15$ ns ± 0.1 ns).[15] The accuracy of the fit of the single decays, as judged by reduced chi-squared (χ^2_R), the autocorrelation function *C*(*j*) of the residuals and the Durbin-Watson parameter (DW), was always acceptable yielding values of $\chi^2_R < 1.2$ and DW > 1.8, respectively.

4.5 Fit of the absorption spectra of 1

After conversion to the energy (wavenumber) scale, the structured BDP-localized absorption band is modeled from the main and the two sub bands of **2** in the respective solvents (employing Gaussian functions). Then, a fourth component (Gaussian-type) is included to describe the CT band.

4.6 Determination of pK_a

The pK_a (±0.1) of **1** was derived from fluorometric titrations ($N = 3$, excitation at the isosbestic point at 630 nm, emission monitored at 656 nm) in an ethanol–water 1 : 1 (v/v) mixture[5] by employing the Henderson–Hasselbalch equation $pK_a = pH - \log(I_F^{max} - I_F)/(I_F - I_F^{min})$.

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References

1 R. P. Haugland, *Handbook of Fluorescent Probes and Research Chemicals*, 6th edn., Molecular Probes, Eugene, OR, 1996.

2 E. Vos de Wal, J. Pardoen, J. A. van Koeveringe and J. Lugtenburg, *Recl. Trav. Chim. Pays-Bas*, 1977, **96**, 306; J. Karolin, L. B.-Å. Johansson, L. Strandberg and T. Ny, *J. Am. Chem. Soc.*, 1994, **116**, 7801.

3 (*a*) L. H. Thoresen, H. Kim, M. B. Welch, A. Burghart and K. Burgess, *Synlett*, 1998, 1276; (*b*) H. Kim, A. Burghart, M. B. Welch, J. Reibenspies and K. Burgess, *Chem. Commun.*, 1999, 1889; (*c*) A. Burghart, H. Kim, M. B. Welch, L. H. Thoresen, J. Reibenspies, K. Burgess, F. Bergström and L. B.-Å. Johansson, *J. Org. Chem.*, 1999, **64**, 7813; (*d*) J. Chen, J. Reibenspies, A. Derecskei-Kovacs and K. Burgess, *Chem. Commun.*, 1999, 2501; (*e*) J. Chen, A. Burghart, A. Derecskei-Kovacs and K. Burgess, *J. Org. Chem.*, 2000, **65**, 2900.

4 For recent reviews, see: A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; A. P. de Silva and A. J. M. Huxley, in *Applied Fluorescence in Chemistry, Biology and Medicine*, ed. W. Rettig, B. Strehmel, S. Schrader and H. Seifert, Springer, Berlin, 1999, p. 179; and various contributions to *Near-Infrared Dyes for High Technology Applications*, ed. S. Dähne, U. Resch-Genger and O. S. Wolfbeis, Kluwer Academic, Dordrecht, 1998.

5 For a more general introduction to fluorescent pH probes, see: M. Maus and K. Rurack, *New J. Chem.*, 2000, **24**, 677.

6 (*a*) M. Kollmannsberger, T. Gareis, S. Heinl, J. Breu and J. Daub, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1333; (*b*) T. Gareis, C. Huber, O. S. Wolfbeis and J. Daub, *Chem. Commun.*, 1997, 1717; (*c*) M. Kollmannsberger, K. Rurack, U. Resch-Genger and J. Daub, *J. Phys. Chem. A*, 1998, **102**, 10211; (*d*) K. Rurack, M. Kollmannsberger, U. Resch-Genger and J. Daub, *J. Am. Chem. Soc.*, 2000, **122**, 968.

7 J. Herbich and A. Kapturkiewicz, *J. Am. Chem. Soc.*, 1998, **120**, 1014; M. Maus, W. Rettig, S. Depaemelaere, A. Onkelinx, F. C. De Schryver and K. Iwai, *Chem. Phys. Lett.*, 1998, **292**, 115; M. Maus, W. Rettig, D. Bonafoux and R. Lapouyade, *J. Phys. Chem. A*, 1999, **103**, 3388.

8 T. Werner, C. Huber, S. Heinl, M. Kollmannsberger, J. Daub and O. S. Wolfbeis, *Fresenius' J. Anal. Chem.*, 1997, **359**, 150.

9 H. Shizuka, T. Ogiwara and E. Kimura, *J. Phys. Chem.*, 1985, **89**, 4302.

10 W. Hinz, R. A. Jones and T. Anderson, *Synthesis*, 1996, **8**, 620.

11 D. Magde, J. H. Brannon, T. L. Cremers and J. Olmsted III, *J. Phys. Chem.*, 1979, **83**, 696.

12 D. F. Eaton, *Pure Appl. Chem.*, 1988, **60**, 1107.

13 U. Resch and K. Rurack, *Proc. SPIE–Int. Soc. Opt. Eng.*, 1997, **3105**, 96.

14 N. Boens, N. Tamai, I. Yamazaki and T. Yamazaki, *Photochem. Photobiol.*, 1990, **52**, 911.

15 W. R. Ware, M. Pratinidhi and R. K. Bauer, *Rev. Sci. Instrum.*, 1983, **54**, 1148.