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Chiral discrimination with a fluorescent boron–dipyrromethene dye

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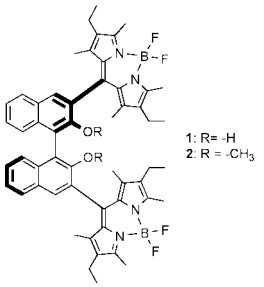
The optically active binaphthalene boron–dipyrromethene (BDP) conjugate 1 shows chiral discrimination towards the enantiomers of 1-phenylethylamine by distinguishable quenching rates of the BDP fluorescence.

The chiral discrimination between enantiomers during molecular recognition processes[1] is of great importance in many fields of pharmaceutical chemistry, bio- and food technology.[2] For instance, the knowledge of the stereochemical structure of a potential drug is essential for rational and successful drug design as potency, selectivity and bioavailability can differ markedly for enantiomers of a chiral compound.[3] Solution-based sensor systems can be an important aid in these development processes,[4] since they allow the study of the processes of drug–receptor interactions. A functionalization with chromogenic[5] or fluorescent[6] reporter molecules facilitates the application of spectrophotometric and fluorometric analytical techniques that offer many advantages for highly sensitive chiral recognition. The use of steady-state and time-resolved fluorometry especially can improve rapid and quantitative analysis and is well-suited for high-throughput screening.[7] In our approach towards efficient enantioselective fluorescent sensor molecules, we relied on the versatile[8,9] and, when equipped with an appropriate receptor, efficiently chemically switchable[9] boron–dipyrromethene (BDP) fluorophore and designed the enantiomerically pure BDP conjugate **1**. 1,1′-bi-2-naphthol, known to selectively recognize optically active amines or amino acids,[5d,10] was chosen by us as the chiral receptor unit. In this communication, we report on the ability of **1** to differentiate between (*R*)- and (*S*)-1-phenylethylamine by specific modulation of its fluorescence signal at wavelengths > 500 nm.

The optically active *R*-enantiomers of **1** and the reference



compound **2** were obtained from enantiomerically pure 1,1′-binaphthalene carbaldehydes[11a,b] and 2,4-dimethyl-3-ethylpyrrole by literature procedures.[11c,d]

In agreement with earlier studies on a phenol-appended BDP dye,[9a] deprotonation of the hydroxy groups of **1** leads to a strong decrease of the fluorescence (Φ_f^1 = 0.62), most probably involving a photoinduced electron transfer-like quenching mechanism. Fig. 1 shows this 'switching OFF' of the BDP emission at 542 nm for a microtitration of **1** with diisopropylethylamine in acetonitrile.† Whereas the emission band position does not change during the titration, the BDP $S_1 \leftarrow S_0$ absorption band shifts slightly to the blue (from 523 to 521 nm). For **2**, where the hydroxy groups are exchanged with methoxy groups, no spectral shifts occurred in either, absorption (526 nm) or emission (542 nm, Φ_f^2 = 0.80), and only at comparatively high amine concentrations (*ca.* $> 10^{-2}$ M) was a slight reduction in fluorescence observed. To get better insight into the nature of the quenching process(es) involved, time-resolved fluorescence titrations were carried out. In the case of purely static quenching, *i.e.* when a non-fluorescent ground-state complex is formed, the fluorescence lifetime remains constant (τ_f = const.) and only the amplitude is gradually decreased during the titration ($a \neq$ const.). When dynamic quenching occurs, the number of emitting molecules is not changed (a = const.) but the lifetime is reduced ($\tau_f \neq$ const.) with increasing quencher concentration as a result of an increase in collisional frequency of an excited fluorophore with a quencher. Time-resolved quenching experiments with diisopropylethylamine revealed single exponential decays for both dyes. However, whereas for **2** the amplitude of the 6.17 ns component does not change, in the case of **1** an increase in base concentration leads to a concomitant decrease of the amplitude of the 5.15 ns decay species. At high amine concentrations, a slight but gradual decrease of the fluorescence lifetime is found for **1** and **2**, with an unchanged amplitude in the case of **2**. These results, along with the spectrophotometric changes, suggest that the reaction of diisopropylethylamine with **1** leads to the formation of an entirely non-fluorescent, deprotonated species of **1**, *i.e.* static quenching occurs, the respective K_S being determined to 1.1×10^4 M^{-1}.‡ Moreover, at high concentrations, base-induced dynamic quenching seems to be responsible for a reduction in fluorescence decay time for both, the sensor molecule as well as the reference compound. Reprotonation by TFA restored the initial fluorescence intensity.

These findings, along with our recent investigations on the transfer of chiral information from the optically active 1,1′-binaphthalene skeleton to the appended BDP-chromophores,[12] encouraged us to test the ability of **1** for chiral discrimination of optically active bases, (*R*)-(+)- and (*S*)-(–)-1-phenylethylamine (*R*-/*S*-PEA). In analogy to the results reported in the preceding

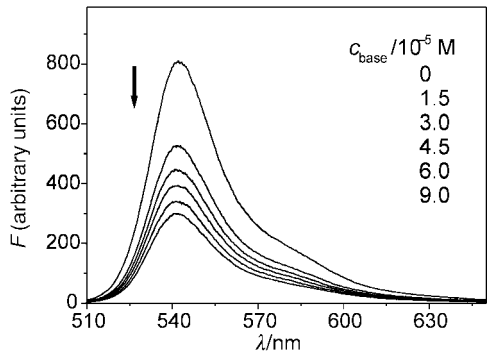


Fig. 1 Decrease in BDP fluorescence upon addition of diisopropylethylamine to a 5×10^{-6} M solution of **1** in acetonitrile; excitation at 490 nm.

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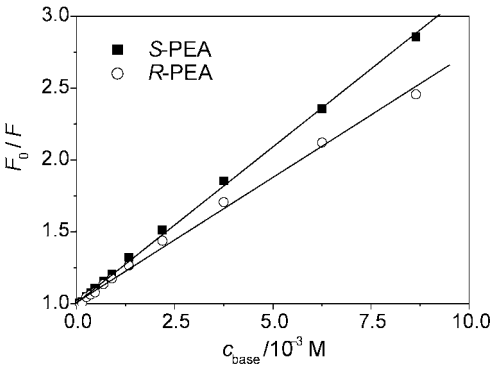

Fig. 2 Plots for the quenching of **1** with *S*-PEA and *R*-PEA in acetonitrile ($c_1 = 5 \times 10^{-6}$ M, excitation/emission at 490/540 nm, $r = 0.999/0.998$ for *S*-PEA/*R*-PEA).

paragraph, an interaction between a chiral quencher and the 1,1′-binaphthalene receptor unit should be detectable by specific changes of the BDP fluorescence signal. Upon addition of *R*- and *S*-PEA respectively, a decrease of the intensity of the fluorescence band was observed (Fig. 2), the emission maximum remaining virtually unchanged. The hypsochromic shifts in absorption (to 518 and 519 nm for *R*-PEA/**1** and *S*-PEA/**1**) are twice as large as those measured for the achiral bases. In addition, the molar absorptivities increase, the presence of *R*-PEA resulting in a stronger effect. The fluorescence lifetimes of **1** are unaffected by *R*- and *S*-PEA, and only at amine concentrations $>10^{-2}$ M does the excess of base lead to bimolecular, diffusion-controlled quenching. Analogous studies of **2** revealed that the fluorescence signal shows no comparable response upon addition of PEA, except for the dynamic quenching. This indicates that the hydroxy groups are essential for an interaction between the chiral analytes and the binaphthalene BDP conjugate, entailing specific modulations of the spectroscopic signal. Enantiomer-specific changes in the absorption spectra of 1,1′-binaphthol–amine associates have been reported before[13] and have been attributed to the formation of differently structured diastereomeric complexes,[13,14] most probably involving (more or less) strongly hydrogen-bound ground-state complexes (R–OH$\cdots$NR$_3$) or ion pairs of RO$^-\cdots$HNR$_3^+$-type. In the case of **1**, such differences in binding of *R*- and *S*-PEA can also result in a structural reorientation of the BDP chromophores. A better verification of the nature of the sensor molecule–analyte interaction/complex formation was not possible with the methods employed here, because several BDP-localized transitions which are centered in the region of the binaphthyl absorption hampered a closer inspection of the spectroscopic changes taking place directly at the binding/reaction site.[13]

In order to examine the efficiency of the quenching process for *R*- and *S*-PEA, the experimental data were analysed according to the equation for static quenching.‡ Fig. 2 shows the corresponding plots for the quenching of **1** with *R*- and *S*-PEA. The different slopes clearly indicate the suitability of **1** as a chirally discriminating sensor for optically active amines. The steeper slope for quenching with *S*-PEA, based on a higher $K_S = 226$ M^{-1} for *S*-PEA–**1** as compared to $K_S = 161$ M^{-1} for *R*-PEA–**1**, suggests that association of the *S*-enantiomer and **1** is more efficient. The ratio of $K_S(R–S)/K_S(R–R) = 1.40$ is comparatively high for amine complexes of a simple binaphthol receptor unit in acetonitrile[10*f*,*g*,13] and clearly stresses the potential of **1** as an enantioselective sensor molecule.

In conclusion, we have shown that the combination of the chiral recognition features of a substituted 1,1′-binaphthalene unit with the favourable spectroscopic properties of the BDP chromophore presents a promising approach towards 'ON'–'OFF' signaling of chiral analytes, advantageous both in spectral region and time domain.

Notes and references

† For the equipment used and general experimental procedures including fluorescence quantum yield determination, see ref. 9*b*. All quantum yields and lifetime data were obtained at 295 K, the uncertainties of the quenching constants are $< \pm 4\%$ ($n = 4$).

‡ If fluorescence quenching is caused by static as well as dynamic interactions of sensor and analyte molecules, the single quenching constants can in principle be separated by considering time-resolved emission data (for details, see J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Plenum, New York, 1983, p. 260). In the case of **1**, dynamic quenching was only observed at very high concentrations of the corresponding bases. The quenching of the BDP fluorescence was nearly complete at much lower base concentrations due to static interactions, *i.e.* deprotonation (for diisopropylethylamine) or association (for the chiral amines) reactions. Therefore, the static quenching constants K_S reported here could readily be determined according to $F_0/F = 1 + K_S c_Q$, where F_0 is the fluorescence yield in the absence of quencher Q, F is the respective parameter in the presence of Q, c_Q is the concentration of the quencher and K_S is the association constant, in a concentration range where no reduction in fluorescence lifetime was observed. In all of these cases, plots of F_0/F *vs.* c_Q were strictly linear with correlation coefficients $r > 0.99$.

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