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Molecular Multi-Wavelength Optical Anion Sensors

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Polychromatic fingerprinting of simple anions (halides, oxo anions) is achieved by employing neutral and charged multicolor fluorescent probes based on ferrocene-spaced dansyl and naphthyl groups (**1**/**1**$^+$; **2**/**2**$^+$). The conformation of the neutral double dye sensor **2** has been elucidated by NMR spectroscopic techniques (in solution), by X-ray crystallography (solid state) and by DFT calculations (gas phase). The double-dye receptors **2**/**2**$^+$ exhibit specific emission responses in the presence of anions X^- when excited at the absorption maxima of the dyes (fingerprint).

Introduction

In recent years, great efforts were made in the area of anion binding and sensing.[1] One well-established principle in the design of artificial anion sensors is the binding site/signalling subunit approach. The binding site acts as a host, usually with a preorganized architecture for the anion as the guest. Amides as good hydrogen-bond donors and hydrogen-bond acceptors have shown to be versatile building blocks for neutral (oxo) anion binding sites.[2] The signalling unit consists of a redox-active, chromogenic or fluorogenic group linked to the receptor, which allows sensing of anions with electrochemical, colorimetric or fluorimetric methods. A particular highlight in the field of anion sensing are compounds based on ferrocenes as electrochemically robust building blocks.[3] Electrochemical detection is based on cathodic shifts in the redox process of ferrocenyl units in the host–guest molecule, which has been successfully employed by Astruc, Kraatz and others.[4] Further development of ferrocenyl-based sensors utilizes the combination with a fluorescent signalling subunit. In the absence of an analyte, the ferrocenyl unit largely quenches the emission of the fluorescent chromophore by photoinduced electron transfer (PET) or energy transfer.[5] Beer, Molina, Tárraga and other groups have shown that formation of the host–guest complex can partially restore the luminescence, which can be exploited to detect anions (or cations) by a fluorimetric turn-on signal.[6] Usually only a single signal (on/off) or a ratiometric signal change (one signal on, one signal off) is observed for a specific analyte. Thus, discrimination between different but similar anions is difficult to achieve with a single receptor.

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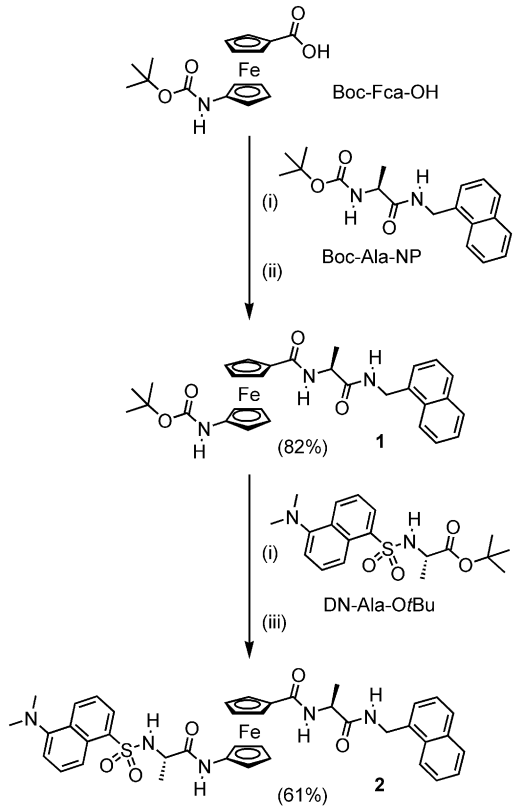
We envisaged a multi-wavelength turn-on fluorescent molecular probe with possible chiral discrimination. To install the multiwavelength detection option we employ *two* chromophores with different excitation and emission profiles. Before analyte binding the fluorescence of these dyes should be largely quenched, and analyte binding should restore the dye emission. The fluorescence quenching will be provided by a ferrocene unit.[5] The binding site should be flexible enough to allow sensing of anions of different size and shape. This feature will also be provided by the flexible ferrocene hinge.[7] Incorporation of chiral elements (preferably from the chiral pool) should principally allow enantiodifferentiation, although this is not the primary goal of this proof-of-principle study.

Results and Discussion

We have covalently assembled all these ingredients – two complementary multiwavelength dyes, a quencher unit, a flexible binding site, chirality – in ferrocene peptide **2** (Scheme 1). Ferrocene amino acid (1′-aminoferrocene-1-carboxylic acid, Fca)[8] has been functionalized at its *C*-terminus with a chiral *N*-(1-naphthylmethyl)alanyl chromophore (H-L-Ala-NP) and at its *N*-terminus with a chiral *N*-dansylalanyl chromophore (DN-L-Ala-OH) to give the organometallic peptide DN-Ala-Fca-Ala-NP (**2**). The truncated intermediate Boc-Fca-Ala-NP (**1**) without the DN chromophore fragment (DN-Ala) has been studied for comparison.

Recently, two mixed sensors have been applied to the ratiometric detection of Zn^{2+} ions by using a combination of FRET (fluorescent resonant energy transfer) and PET communication channels.[9] A covalently linked DN/NP FRET pair has been successfully utilized in monitoring a sugar-transfer reaction (NP: λ_{exc} = 280 nm; λ_{emiss} = 340 nm; DN: λ_{exc} = 340 nm; λ_{emiss} = 540 nm) and a base-catalyzed hydrolysis reaction (NP: λ_{exc} = 285 nm; λ_{emiss} = 338 nm; DN: λ_{exc} = 340 nm; λ_{emiss} = 500 nm).[10] The covalently



Scheme 1. Synthesis of peptidic ferrocene receptors **1** and **2**. (i) TFA, CH_2Cl_2; (ii) HOBt, HBTU, NEt_3, CH_2Cl_2; (iii) HOBt, DCC, CH_2Cl_2.

linked DN/NP pair in **2** might also communicate directly with each other through space by FRET (the distance is well below the Förster radius, see below) or the fluorescence properties might be influenced by the connecting ferrocene unit.

Weak emission is expected for compounds **1** and **2**, since ferrocenyl residues are known to quench luminescence by either photoinduced electron transfer (PET) from the ferrocene to the excited dye or by energy transfer.[5] When excited at 284 nm, the NP derivative **1** displays a weak fluorescence signal around 335 nm at room temperature in CH_2Cl_2 corresponding to the naphthalene monomer emission (quantum yield about 1.4% of that of the reference dye Boc-Ala-NP). Similarly, the DN emission quantum yield of **2** (λ_{exc} = 340 nm, λ_{obs} = 506 nm) is reduced to about 6.6% as compared to that of reference compound DN-Ala-O*t*Bu lacking the ferrocene quencher. When receptor **2** is irradiated at λ_{exc} = 284 nm, dual emission is observed from NP and from DN, which might be due to either direct excitation of DN at 284 nm or to FRET from NP to DN or to a combination of both mechanisms. A 1:1 mixture of the chromophores Boc-Ala-NP/DN-Ala-O*t*Bu in CH_2Cl_2 excited at 284 nm shows a structured emission of NP at λ = 326/337 nm and an emission of DN at λ = 500 nm with an intensity ratio of 1:2.4. In **2** the corresponding ratio amounts to 1:4.2, which might be due to FRET from NP to DN or to different quenching efficiencies of NP and DN by the connecting ferrocene unit. Irrespective of the modulation mechanisms involved **2** exhibits dual emission (from NP and from DN).

The preferred conformation of the two-armed host **2** has been elucidated by theoretical calculations (DFT),[11] NMR investigations in CD_2Cl_2[12] and finally by single-crystal X-ray structure analysis[13] (Figure 1 and Supporting Information). Essentially, all methods yield the same spatial arrangement of the two chiral dye-modified arms, which is determined by two intramolecular hydrogen bonds from the NH groups adjacent to the ferrocene unit to the CO or SO groups of the neighboring substituent.[11–13] Analogous conformations have been observed previously for AA-Fca-AA bioconjugates lacking aromatic chromophores (AA = α-amino acids).[14] The DN···NP distance (estimated as C21···C31) can be varied from 6 to 10 Å by rotation around single bonds according to models with the double intramolecular hydrogen bonds remaining intact. Models without the double hydrogen bond suggest a maximum distance of the dyes of around 16 Å by rotation of the cyclopentadienyl rings. All these distances are well below the Förster radius of the chromphore pair.[15]

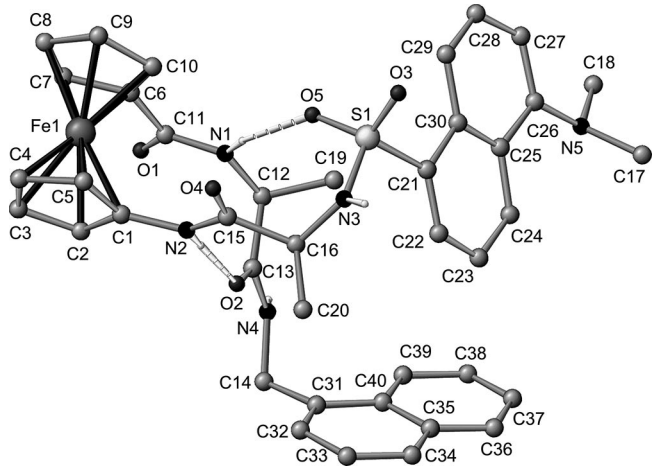


Figure 1. Molecular structure of **2** in the $P2_12_12_1$ crystal and atom numbering (CH hydrogen atoms omitted; intramolecular hydrogen bonds indicated by dashed lines).

Analyte binding is expected to disrupt this folded structure and to induce refolding of the ferrocenepeptide around the target and possibly also association mediated by the analyte.[7] The conformational reorganization should modify energy- and electron-transfer pathways in some way so that the binding event is translated into different optical signals. Anions X^- of different sizes and shapes (spherical, tetrahedral, trigonal planar: F^-, Cl^-, Br^-, HSO_4^-, NO_3^-, $H_2PO_4^-$, L/D-Ac-Ala) were added as their tetra-*n*-butylammonium salts to CH_2Cl_2 solutions of **1** or **2**.

ESI-negative experiments show that indeed anions X^- coordinate to the receptors **1** and **2** as peaks of the respective $[\mathbf{1} + X]^-$ and $[\mathbf{2} + X]^-$ ions are observed at expected *m*/*z*

values, e.g. at m/z = 652 [**1** + H_2PO_4]$^-$, 685 [**1** + Ac-Ala-O]$^-$; 794 [**2** + Cl]$^-$, 838 [**2** + Br]$^-$ or 821 [**2** + NO_3]$^-$ (see Supporting Information for detailed spectra).[14d,16]

In the presence of anions, the emission intensity of **1** changes at λ_{obs} = 335 nm (NP, λ_{exc} = 284 nm) with 4.8× enhancement for fluoride and unspectacularly for the other ions (0.8× to 1.8×; see Supporting Information). For F^-, $H_2PO_4^-$ and HSO_4^-, an excimer emission band is observed around 480 nm[17] suggesting that (at least) two receptors **1** are connected by these anions linking two naphthalene units. Indeed, Job plots indicate a 2:1 complex of **1** and $H_2PO_4^-$ but a 1:1 complex of **1** and Cl^- (see Supporting Information). Interestingly, a 1:2 complex is found for **1** and F^-, which might be explained by the strong basicity of fluoride leading to deprotonation of amide protons as has been observed previously for similar systems.[6c,6d,17]

Slightly higher enhancement factors at λ_{obs} = 335 nm (7.4× for X^- = $H_2PO_4^-$ and 5.6/5.2× for L/D-Ac-Ala-O$^-$) are observed when **2** is irradiated at λ_{exc} = 284 nm in the presence of X^-, probably due to the presence of more potential hydrogen-bonding groups (hydrogen-acceptor and -donor groups) in host **2** (see Figure 2, top, for selected anions; see Supporting Information for all anions). However, no discrimination is achieved on the basis of this single measurement.

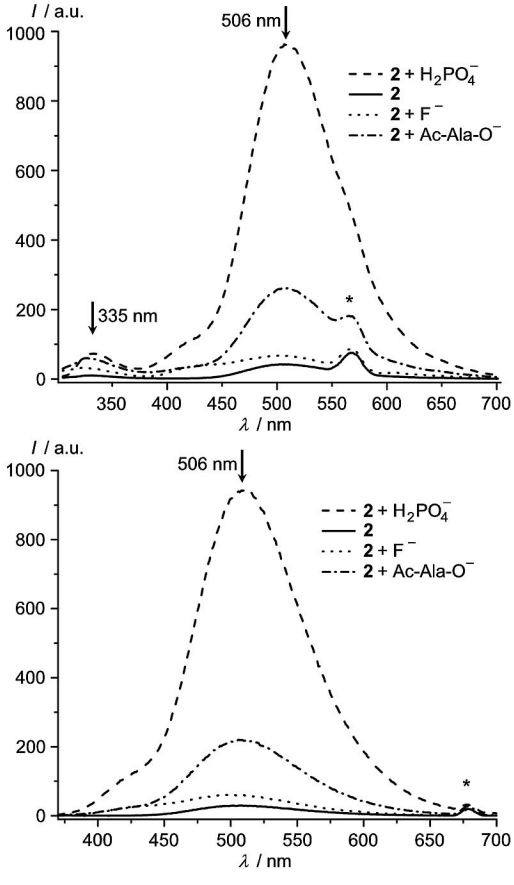


Figure 2. Emission spectra of **2** in CH_2Cl_2 and in the presence of $H_2PO_4^-$, F^- and Ac-Ala-O$^-$ upon irradiation at λ_{exc} = 284 nm (top) and at λ_{exc} = 340 nm (bottom) (the asterisks correspond to 2× λ_{exc}).

As **2** exhibits dual emission, the fluorescence of the DN chromophore was simultaneously monitored at λ_{obs} = 506 nm upon excitation at λ_{exc} = 284 nm with enhancement factors ranging from 1.1 to 23.0× (Figure 2, top). The DN unit of **2** can also be excited directly at λ_{exc} = 340 nm (Figure 2, bottom), and quite large fluorescent enhancements are observed for $H_2PO_4^-$ (31.8×), HSO_4^- (8.2×) and L/D-Ac-Ala$^-$ (7.5/7.3×) at λ_{obs} = 506 nm (Figure 3). Job plots indicate 1:1 complexes of **2** and Cl^- and $H_2PO_4^-$, respectively (see Supporting Information). The chloride binding affinity of receptor **2** was exemplarily studied by using ^{1}H NMR titration techniques with tetrabutylammonium chloride in CD_2Cl_2. The stability constant was elucidated by using non-linear least-squares fitting of the chemical shifts of the four amide protons of **2**. The 1:1 binding constant of receptor **2** and chloride was determined as K = 730 M^{-1} at 25 °C in CD_2Cl_2 [see Supporting Information for graphical representations of the binding isotherms of all NH protons; NH^1: K = 738(87) M^{-1}; NH^2: K = 765(83) M^{-1}; NH^3: K = 749(89) M^{-1}; NH^4: K = 670(68) M^{-1}; $R^2 > 0.995$ in all cases], fully comparable to those of other oligoamide-based anion receptors.[1,6,7] Job's analysis of the **2**/F^- system is ambiguous due to competing deprotonation of **2**.[6c,6d,17]

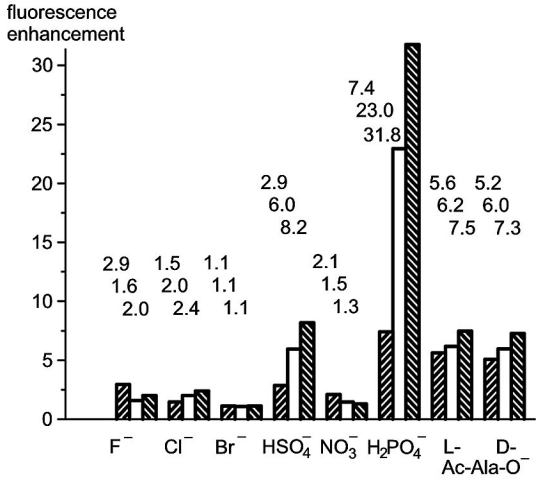


Figure 3. Fingerprint of fluorescence enhancement factors of **2** upon addition of anions F^-, Cl^-, Br^-, HSO_4^-, NO_3^-, $H_2PO_4^-$, L-Ac–Ala-O$^-$, D-Ac–AlaO$^-$ by using different excitation and observation wavelengths (from left to right: $\lambda_{exc}/\lambda_{obs}$ = 284/335; 284/506; 340/506 nm).

Binding of $H_2PO_4^-$ to a ruthenium(II)–bipyridyl–bis-(amidoferrocene) receptor has also been shown to reduce the intramolecular luminescence quenching by ferrocene and to promote the ^{3}MLCT emission of the Ru^{II}–polypyridyl chromophore.[6a] A similar mechanism might be operative in ferrocene–DN conjugate **2**. In total, a fingerprint of three relative intensity values per anion ($\lambda_{exc}/\lambda_{obs}$ = 284/335, 284/506, 340/506 nm) is easily acquired. This triple is different for each anion investigated (Figure 3). Especially $H_2PO_4^-$, HSO_4^- and L/D-Ac-Ala$^-$ are well discriminated from the halides and nitrate.

Both receptors **1** and **2** can be reversibly oxidized to the corresponding ferrocenium–peptides $\mathbf{1}^+$ and $\mathbf{2}^+$ at $E_{1/2}$ = 50 mV ($\mathbf{1}/\mathbf{1}^+$) and $E_{1/2}$ = 105 mV ($\mathbf{2}/\mathbf{2}^+$) vs. Fc/Fc^+. The dimethylamino group of the DN moiety in **2** is irreversibly oxidized at higher potential ($E_{1/2}$ = 510 mV; see Supporting Information). It is expected that oxidation of **1** and **2** increases the anion affinity and possibly also affects the fluorescence characteristics of the appended dyes. Indeed, partial fluorescence recovery of appended dyes has been previously observed upon oxidizing Fc to Fc^+ in ferrocene–naphthalimide conjugates (3.8×) or ferrocene–porphyrin conjugates (1.65×).[18] This effect is thought to be due to blocking electron- and energy-transfer pathways, as spectral overlap between Fc^+ absorption and dye emission is very low. However, ferrocenium ions are strong electron acceptors so that electron transfer from the excited dye to Fc^+ might be possible. This PET has been considered inefficient, as it should be highly exergonic und thus kinetically disfavoured (Markus inverted region).[18b]

Preparative oxidation of **1** to $\mathbf{1}^+$ and **2** to $\mathbf{2}^+$ has been achieved by adding silver tetrafluoroborate[19] to a solution of **1** or **2** in CH_2Cl_2. As expected green solutions containing **1** or **2** and $AgBF_4$ display the characteristic ferrocenium absorption band [λ_{max} = 825 nm ($\mathbf{1}^+$) and λ_{max} = 800 nm ($\mathbf{2}^+$)]. Quantum yields of the cations $\mathbf{1}^+$ and $\mathbf{2}^+$ have increased relative to those of neutral **1** and **2** by factors of 7.7 ($\mathbf{1}^+/\mathbf{1}$; NP) and 1.6 ($\mathbf{2}^+/\mathbf{2}$; DN), respectively.

In the presence of the various anions X^-, receptor $\mathbf{1}^+$ shows unimpressive NP fluorescence enhancement from 1.1 to 2.0× (at $\lambda_{exc}/\lambda_{obs}$ = 284/335 nm, see Supporting Information). For the multiwavelength receptor $\mathbf{2}^+$ and X^-, the fingerprint triples based on NP and DN excitation/emission ($\lambda_{exc}/\lambda_{obs}$ = 284/335, 284/506, 340/506 nm) are shown in Figure 4. The triples for receptor $\mathbf{2}^+$ show some more spread from 0.8 to 3.5× as compared to the single factors of $\mathbf{1}^+$ (see Supporting Information).

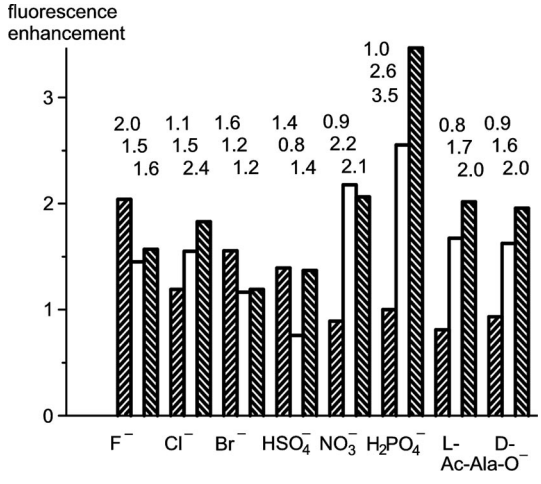


Figure 4. Fingerprint of fluorescence enhancement factors of $\mathbf{2}^+$ upon addition of anions F^-, Cl^-, Br^-, HSO_4^-, NO_3^-, $H_2PO_4^-$, L-Ac–Ala-O^-, D-Ac–AlaO^- by using different excitation and observation wavelengths (from left to right: $\lambda_{exc}/\lambda_{obs}$ = 284/335, 284/506, 340/506 nm).

Six relative-intensity changes for each anion X^- can be easily determined by using receptors **2** and $\mathbf{2}^+$ and different excitation and observation wavelengths (λ_{exc} = 284, 340 nm; λ_{obs} = 335, 506 nm), namely *I*(284/335 nm; **2**), *I*(284/506 nm; **2**), *I*(340/506 nm; **2**) and *I*(284/335 nm; $\mathbf{2}^+$), *I*(284/506 nm; $\mathbf{2}^+$), *I*(340/506 nm; $\mathbf{2}^+$). Gratifyingly, the intensity patterns are sufficiently different for the anions investigated allowing for example to distinguish chloride from fluoride or HSO_4^- from $H_2PO_4^-$ (Figures 3 and 4). Only the enantiodifferentiation between L- and D-Ac–Ala^- is not yet significant (Figures 3 and 4). Thus, all seven anions can be discriminated and distinguished by using a single receptor **2** and its charged counterpart $\mathbf{2}^+$ and six relative fluorescence intensities.

The exact binding modes of the anions and the mechanism of fluorescence modulation are still speculative and obviously different for different anions (Job's analyses). However, it is evident that analyte binding changes FRET and PET pathways in different manners within ferrocenepeptide **2** and oxidized ferrocenepeptide $\mathbf{2}^+$, which allows to differentiate the seven anions.

Conclusions

The six-parameter polychromatic fingerprinting using only a single basic molecular compound **2** and its positively charged congener $\mathbf{2}^+$ could pave the way for multianion analysis in competitive assays. In principle, any other chromophore pair with differing absorption/emission patterns can be used. Also enantiospecific anion discrimination might be possible by employing sterically discriminating α-amino acids as constituents of derivatives of $\mathbf{2}/\mathbf{2}^+$. The combinatorial syntheses of such organometallic dye conjugates on solid phases[20] and the exploitation of their fluorescence signaling option towards anion, peptide and protein sensing[21] are subject to current and future work in our group.

Experimental Section

Experimental, spectroscopic and DFT details are given in the Supporting Information.

Supporting Information (see footnote on the first page of this article): Experimental, spectroscopic and DFT details.

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[11] Several conformations of a model of **2** (methyl groups of DN replaced by hydrogen atoms) have been optimized at the DFT/B3LYP (LanL2DZ) level of theory. Two of them with two intramolecular hydrogen bonds *between* the arms are found lowest in energy. They only exhibit a different stereochemistry at the sulfur atom, and they differ in energy by less than 5 kJ mol^{-1}. The intramolecular N1H···O5S1 hydrogen bond (N1···O5 2.87/3.00 Å) is calculated longer than the intramolecular N2H···O2C13 hydrogen bond (N2···O2 2.85/2.82 Å). A conformation with one hydrogen bond *between* the arms and one hydrogen bond *within* one arm is calculated higher in energy (16 kJ mol^{-1}); all conformations with two hydrogen bonds *within* the arms are calculated higher in energy (> 30 kJ mol^{-1}). For details see Supporting Information.

[12] NH resonances (NH1, NH2, NH3, NH4) of **2** were assigned by using NOESY and proton correlation NMR spectra. Variable concentration-dependent ^{1}H NMR spectra of **2** in CD_2Cl_2 (c = 1–65 mM) reveal that the NH protons adjacent to the ferrocene moiety (NH1 and NH2) engage in intramolecular hydrogen bonds (weak concentration-dependent chemical shift), whereas the remote NH groups (NH3 and NH4) form hydrogen bonds only at higher concentrations (stronger concentration-dependent chemical shift) indicating intermolecular interactions. In the NOESY spectrum, a cross peak between NH1 and NH2 is observed. These data are consistent with the DFT and X-ray results. For details see Supporting Information.

[13] Two pseudo-polymorphs (orthorhombic $P2_12_12$ and orthorhombic $P2_12_12_1$) have been crystallized from CH_2Cl_2/Et_2O. CCDC-749023 (**2**·¼CH_2Cl_2) and -749024 (**2**) contain the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. They are distinguished by the amount and type of crystal solvent incorporated and the orientations of the aromatic substituents. The latter is characterized by different torsion angles N–CH_2–C_i–C_o and N–S–C_i–C_o indicating substantial flexibility in this part of the molecule. The basic structural hydrogen-bonding motifs with two intra- and two intermolecular hydrogen bonds NH···O are identical, which suggests a stable conserved motif in this part of the molecule. In all cases the intramolecular N1H···O5S1 hydrogen bond (N1···O5 3.18–3.30 Å) is longer than the intramolecular N2H···O2C13 hydrogen bond (N2···O2 2.73–2.84 Å). For details see Supporting Information.

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