Short thesis for the degree of doctor of philosophy (PhD)

Synthesis and investigation of smart fluorophores

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1. Introduction and Aims

One of the core areas of today's material science research is the preparation and investigation of intelligent materials, tools that respond to environmental stimuli similarly to living systems. An exceptionally important group of them is the solvatochromic fluorescent smart materials, which respond to external stimuli by altering the wavelength or intensity of their emitted light. Our group was the first to discover the group of amino isocyanonaphthalenes (ICANs) and still to this day we are pioneers this field. Since the isocyanide (NC) is one of the most versatile functional groups and because of the assumed putrid smell of these compounds (the ICANs are odorless), relatively few researchers are dealing with it, and may have special features that have not been mapped yet, they can quickly gain practical applications. Isocyanides easily form complexes with transient metal ions (eg silver), which can be used in metal ion analysis.

Our aim was to extend the field of application by utilizing other base aromatic rings than naphthalene (biphenyl and acridine) and by changing the relative position of and the amino and isocyano groups. We were looking for new low molecular weight, non-toxic, low-cost compounds suitable for metal ion detection and/or the neutralization of potentially toxic metal ions (eg mercury).

2. New Scientific Results

2.1. Preparation of new, isocyanobiphenil based, blue light-emitting solvatochromic fluorophores

Our aim was to synthesize a novel family of fluorophores with enhanced solvatochromic properties by increasing the distance between the donor (amino) and the acceptor (isonitrile) groups, yielding an increased dipole moment of the molecule. 4-amino-4'-isocyanobiphenyl (ICAB) was obtained by the exchange of the naphthalene ring of ICAN to biphenyl. To further enhance the polarizability, the amino group was mono- and dimethylated resulting 4-isocyano-4'-methylaminobiphenyl (monoMICAB) and 4-dimethylamino-4'-isocyanobiphenyl (diMICAB). 4,4'-diamino-biphenyl (benzidine) was selected as the starting material based on its easy availability, originating from the extensive use of this compound in commercial dyes. The reactions are shown below:

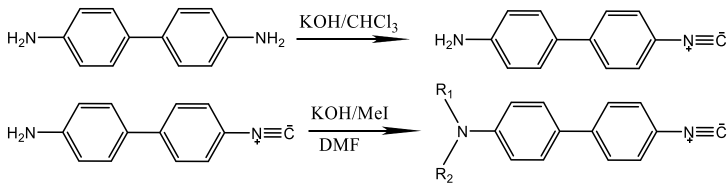

Figure 1.: Synthesis of 4-amino-isocyanobiphenil (ICAB), 4-isocyano-4'-methylaminobiphenyl (monoMICAB) and 4-dimethylamino-4'- isocyanobiphenyl (diMICAB).

In Figure 2 it can be clearly seen that while ICAB has visible emission only in highly polar solvents (DMF, DMSO), monoMICAB has in half of the ones presented, and diMICAB has almost in all of them (except for the most nonpolar, hexane, and only with low intensity in toluene). It should be noted, however, that not only the position of the emission maxima, but the width and shape of the emission band have a great influence on the visible color as well.

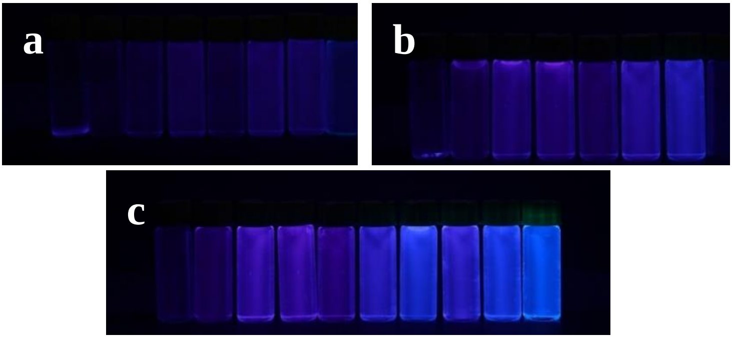

Figure 2.: Light emission of the ICAB (a), monoMICAB (b) and diMICAB (c) series of solutions according to increasing solvent polarity from left to right, excited at λ = 365 nm. The solvents are, in order of polarity, hexane, toluene, dichloromethane, THF, 1,4-dioxane, acetone, acetonitrile, pyridine, dimethylformamide and dimethyl sulfoxide. The concentration of the solutions is 5.6 mg·cm^{-3}

The pictures also demonstrate well the solvatochromic behavior, i.e., the bathochromic shift of the emitted light with increasing solvent polarity. Another important phenomenon can be observed: the quenching of the fluorescence in pyridine in the case of ICAB and monoMICAB, which can be explained by the formation of a hydrogen-bonded complex between the free N - H hydrogen on the fluorophore and a pyridine molecule.

For the mathematical description of the solvatochromic behavior the Lippert-Mataga, Kamlet-Taft and the Catalán (eq. 1) models were applied.

$$\tilde{\nu} = \tilde{\nu}_{max,0} + a_{SA}\mathrm{SA} + b_{SB} \cdot \mathrm{SB} + s_{SP}\mathrm{SP} + t_{SdP}\mathrm{SdP} \quad (1)$$

Using the experimental Stokes shifts ($\Delta\bar{\nu}_{max}$) and by using the corresponding SA, SB, SP and SdP values of the solvents, the Catalán coefficients for $\Delta\bar{\nu}_{max}$ can be obtained by multilinear regression analysis. The values of the coefficients were obtained by multiregression analysis and are found in the equations below.

ICAB

$$\Delta\nu(cm^{-1}) = (-2569 \pm 1990) + (4336 \pm 1009) \cdot SA - (501 \pm 1232) \cdot SB + (9206 \pm 2724) \cdot SP + (3761 \pm 2187) \cdot SdP$$

monoMICAB

$$\nu(cm^{-1}) = (689 \pm 1916) + (3174 \pm 972) \cdot SA - (1065 \pm 1186) \cdot SB + (2347 \pm 2623) \cdot SP + (4891 \pm 2106) \cdot SdP$$

diMICAB

$$\Delta\nu(cm^{-1}) = (-1648 \pm 1964) + (2288 \pm 996) \cdot SA - (1641 \pm 1215) \cdot SB + (4596 \pm 2688) \cdot SP + (3856 \pm 2158) \cdot SdP$$

For better visualization, the measured values of $\Delta\bar{\nu}_{max}$ of the 3 ICABs are plotted in Fig. 3 as a function of their corresponding values calculated.

Figure 3 shows the measured and calculated values of the Stokes–shift for the three compounds. The correlations between the measured and calculated values are good, the regression coefficients of the fitted lines were 0.9 or above. Pyridine values, respectively, had to be disregarded in the evaluation. This may be due to the pyridine-dye complexes, which may be important for further quenching studies.

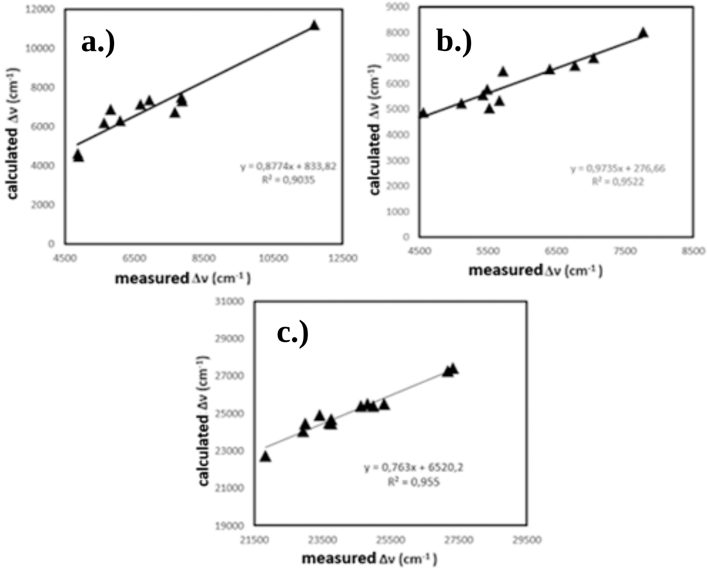

Figure 3: Catalán plots of the Stokes shifts for ICAB (a.), diMICAB (b.), monoMICAB (c.).

Density functional theory was employed to calculate the structures, dipole moments and electronic spectra of the fluorophores in both their ground (GS) and the first excited (ES) states. The obtained dipole moments (μ_{GS} = 8.4 D, μ_{ES} = 19.7 D) were higher than in the case of the previously studied naphthalene derivatives (μ_{GS} = 8.23 D, μ_{ES} = 14.96 D for ICAN), as expected. It is clearly visible in Fig. 4, that the GS of ICAB have a nonplanar structure which is probably due to the single bond character of C1-C10 that allows free rotation between the two aromatic rings.

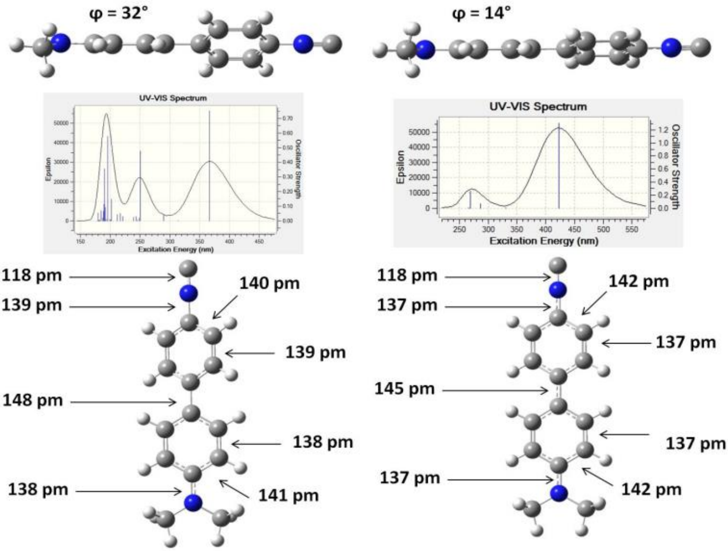

Figure 4.: Results of the DFT structure calculations for diMICAB in hexane.

This and the van der Waals repulsion of the hydrogen atoms results in a relatively large dihedral angle (Φ= 35° for ICAB) which in turn decreases the delocalization between the two aromatic rings. In the excited state the molecule flattens and the change in bond lengths indicates an overall increase in bond order, therefore in conjugation as well. The structures of monoMICAB and diMICAB are similar to that of ICAB: dihedral angles in both the ground state and excited state become slightly lower and the bond lengths are almost the same. The better solvatochromic behavior of the methylated derivates can be explained by their better electron donating ability and as a consequence greater charge separation occurs.

2.2. *Isocyano-aminoacridine based fluorophores, as multifunctional „Swiss-knives”*

The fluorescence properties of ICAAc, monoMICAAc, and diMICAAc for solvents of different polarity are summarized in Figure 5. Independently of solvent polarity, similarly for all three compounds, a broad long wavelength absorption band is seen in the range of ~370–540 nm, which can be attributed to the internal charge transfer transition (ICT) between the donor amino and the acceptor isocyano groups.

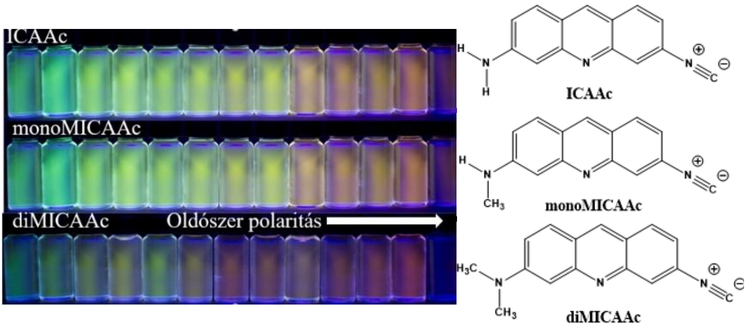

Figure 5.: Structures of 3-amino-6-isocianoacridine (**ICAAc**), 3-N-methylamino-6-isocianoacridine (**monoMICAAc**), 3-N,N-dimethylamino-6-isocianoacridine (**diMICAAc**) (left) and demonstration of the fuorescence properties of the ICAAc derivatives in different solvents (λ_{ex}=365nm). Solvents from left to the right are hexane (1), toluene (2), 1,4-dioxane (3), CH2Cl2 (4), CHCl3 (5), THF (6), MeCN (7), acetone (8), pyridine (9), methanol (10), DMF (11), DMSO (12), water (13).

These shifts are greater than those observed when changing solvent polarity. That is, the higher the electron donating ability of the amino group the higher wavelength the ICT absorption peak is located at. All three dyes have a considerable emission range spanning from green to orange. The emission maximum of diMICAAc in water is the highest of the three dyes with λ_{em} =576 nm, which is 50 nm higher than that of Acridine Orange (AO, λ_{em} =526 nm). In addition, AO has a solvatochromic range of approximately 30 nm, while in the case of monoMICAAc and diMICAAc this value is doubled to more than 60nm.

It is known that acridine based dyes such as AO can be present in different forms in aqueous media. To characterize the acid-base properties of our ICAAc derivatives UV-vis and steady-state fluorescence measurements were carried out in the pH range of 2–12 in the same Britton–Robinson "universal" buffer (BRB). The base (DB) and protonated (DBH^+) forms are presented in Fig. 6.

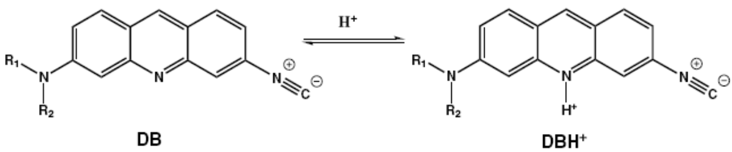

Figure 6.: The protonation equilibrium between the free base form DB and the protonated form DBH^+ of the dyes.

According to Figure 7 the absorption spectra do not change either upon the addition of small amounts of water or base (NH_3). After the addition of acetic acid in ~7 molar excess, a new band starts to appear at ~375 nm and a broad double band structure only appears after the addition of a large excess (~35x molar) of acetic acid. After recording the absorption spectra in the concentration range of $10^{-6} – 10^{-4}$ M in water and in buffers too, no deviation from the Lambert-Beer law was observed, which excludes the presence of dimers. Assuming a simple equilibrium between DB and DBH^+ (Fig. 6) and denoting the pH-dependent property (absorbance or PL intensity) the acid dissociation constant (Ka) can be determined.

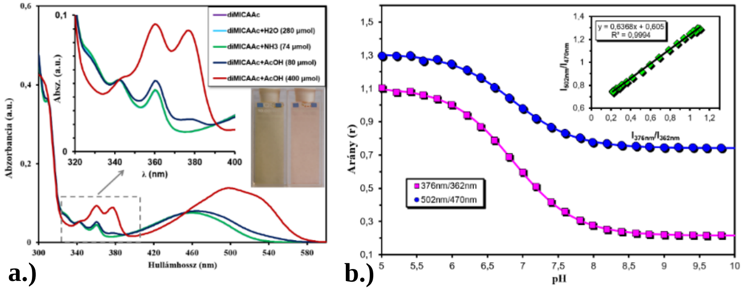

Figure 7.: Changes in the UV spectrum of 3-N, N-dimethylamino-6-isocyanoacridine (diMICAAc) after addition of water, base (NH_3) and acid (CH3COOH). The solvent is acetonitrile. In the inserted figure, the yellow solution (left) belongs to the basic form and the red solution (right) belongs to the acid form.

The PL intensity ratio I_3/I_{11} calculated from the intensities measured at pH=3 and pH = 11 is 2.25 and 1.69 for ICAAc, and monoMICAAc, respectively. The situation is completely different for diMICAAc, where PL intensity increases at higher pH and I_3/I_{11} is only 0.58. Favorably, the pKa value of diMICAAc is 7.5, which falls in the physiological pH range making it a possible candidate for a fluorescent biosensor. For the precise determination of the pH, calibration curves were constructed based on the equation of $r = \frac{b_1+10^{-pH}}{b_2+b_3 10^{-pH}}$

It is known that aromatic amino-isocyano compounds tend to form complexes in the presence of Ag(I) ions and the process is accompanied by significant spectral changes.

2.3. Detection of Hg(II) and Ag(I) with ICAN-based fluorophores

The ICAN derivatives in deionized water were mixed with 3 molar excess of 23 different metal ions covering the whole periodic system and fluorescence spectra were recorded using an excitation wavelength of λ_{ex}=365 nm. Upon the addition of Hg^{2+} the emission maximum shifted from 513 to 456 nm. The excitation maximum belonging to this new maximum was found to be $\lambda_{ex,max}$=337 nm. Consequently, the measurements were repeated using 337 nm excitation (Fig 9).

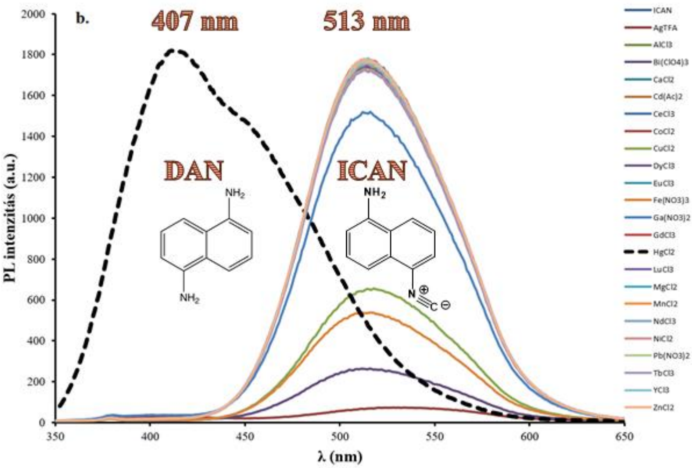

Figure 9: The intensity of ICAN before (λ_{em} = 513 nm) (I_0) and after of the addition of various metal ions (λ_{em} = 406 nm) (I) excited with λ_{ex} = 337 nm.

A completely new peak appeared in the presence of Hg^{2+} with a maximum of λ_{em}=406 nm, where ICAN is virtually nonfluorescent and the intensity increased to 104 times that of the original at 406 nm (Fig. 10 top). More importantly, only the addition of Hg^{2+} yields this huge hypsochromic shift of 102 nm in the spectrum (Fig. 10 bottom), making the method highly selective to Hg^{2+}.

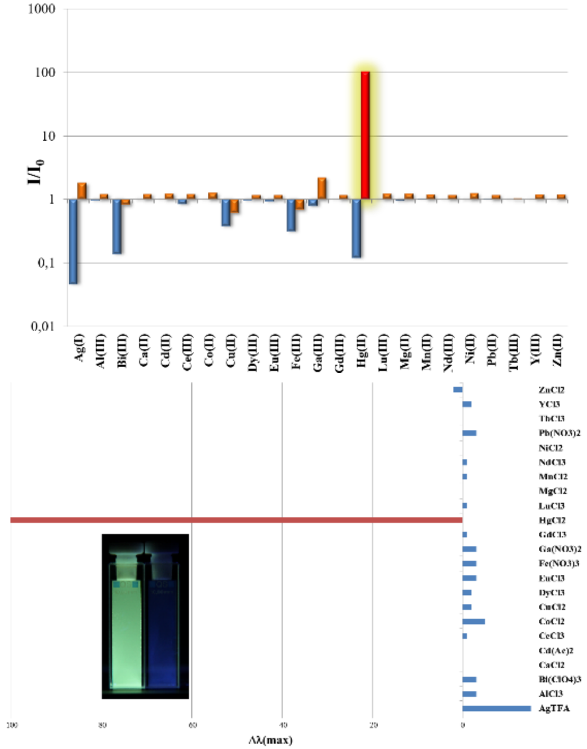

Figure 10.: The emission maxima shift of the ICAN + Me^{n+}. The effect of Hg^{2+} indicated with red column. The inset shows before (left) and after (right) the addition of Hg^{2+}. λ_{ex} = 365 (water: acetonitrile, 96:4 v/v, T = 20 °C, [ICAN] = $4,75 \cdot 10^{-5}$ M, $[Me^{n+}]$ = $1,70 \cdot 10^{-4}$M)

The addition of all the other ions either results no change or a slight bathochromic shift. A few ions (Bi^{3+}, Fe^{3+} and Cu^{2+}) have a moderate quenching effect on the fluorescence of ICAN, probably due to the acidic character of their solutions, or their complexation with the amino group.

The appearance of the two new peaks at 406 nm and 456 nm, respectively, can be attributed to an interaction of ICAN with Hg^{2+}, most probably the reduction of the isocyano group to primary amine. Indeed, the emission spectra of 1,5- diaminonapthalene (DAN), DAN + Hg^{2+} and ICAN + Hg^{2+} are identical with a maximum of 406 nm (Fig 11 a). In addition, the presence of Hg^{2+} has little effect on the spectrum of DAN. The photoluminescence (PL) spectrum of ICAN + Hg^{2+} shows significant pH dependence (Fig 11 b). The spectra of DAN and DAN + Hg^{2+} recorded at different pHs also contain two peaks and the change follows that of ICAN + Hg^{2+}. Consequently, the 456 nm peak may be assigned to a protonated species. To enhance sensitivity pH = 6 was selected for further experiments, because of the absence of the protonated species. The stoichiometry of the reaction was determined using the Job plot (Fig 11 c) and was found to be 1:1 ICAN:Hg^{2+}.

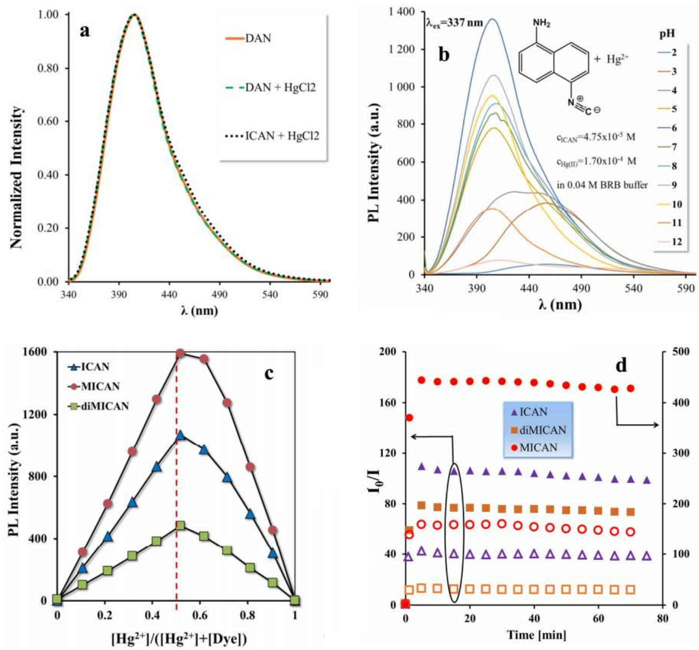

Figure 11.: a) Emission spectra DAN, DAN + $HgCl_2$ and ICAN + $HgCl_2$. b) pH dependence of ICAN + $HgCl_2$. c) Job plot for ICAN, MICAN and diMICAN d) Reaction time and stability for the ICAN-derivates + $HgCl_2$ (Hg^{2+} : ICAN1:1 (n/n) (full dot) and 1: 0,1 (Hg^{2+} : ICAN) n/n (vacant dot).

As can be seen in Fig 11 d the intensity of the amine peak reaches its maximum within 5 minutes for all three ICAN derivatives after the addition of Hg^{2+} at pH = 6, using both 1:1 and 1:0.1 molar ratios, respectively. The intensity variation remains within 10% for at least 70 minutes. The results suggest that this method is quick and stable for Hg^{2+} determination.

2.4. *Effect of substitution position on the photophysical properties of ICAN isomers*

The properties of 1,4-isocyanoaminonaphthalene (1,4-ICAN) and 2,6-isocyanoaminonaphthalene (2,6-ICAN) isomers are discussed in comparison with those of 1,5-isocyanoaminonaphthalene (1,5-ICAN), which exhibits a large positive solvatochromic shift similar to that of Prodan. In these isocyanoaminonaphthalene derivatives, the isocyano and the amine group serve as the donor and acceptor moieties, respectively. It was found that the positions of the donor and the acceptor groups in these naphthalene derivatives greatly influence the Stokes and solvatochromic shifts.

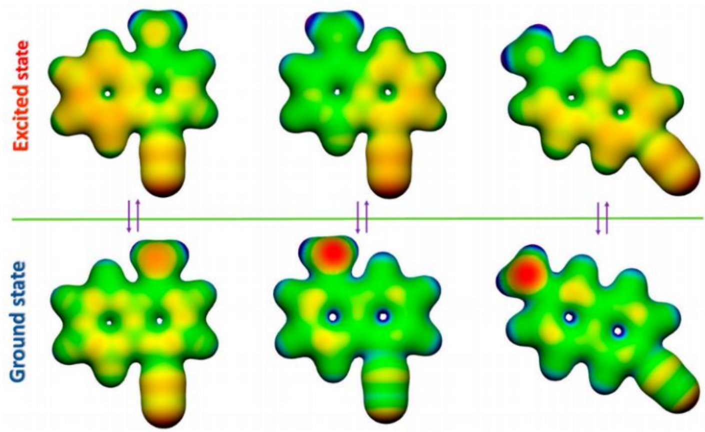

Figure 12.: Electrostatic potential map (ESP) of ICAN derivatives in ground and excited state from left to right1,4-ICAN, 1,5-ICAN and 2,6-ICAN

To gain additional information on the behavior mentioned before the distribution of the electrons was carried out by mapping the electrostatic potential (ESP) of the relaxed ground and excited states as shown in Figure 12. In every case, a large negative charge is centered on the amino nitrogen, which diminishes upon reaching the relaxed excited state. This is also reflected by a change in the geometry as the amino group flattens, or in other words, rehybridizes to an sp^2 state where the nonbonding electrons enter the conjugated π system of the naphthalene ring. The isocyano group; however, shows a different behavior for 1,4-ICAN and the other two molecules. In 1,4-ICAN, a considerable negative charge is centered on this moiety in the ground state already, which changes only slightly upon reaching the relaxed excited state. The 1,5-ICAN and 2,6-ICAN isomers; however, reveal a charge transfer from the donor amino to the acceptor isocyano group, which is located on the far ring. The final electronic configuration of the excited state is reached almost instantaneously after excitation in 1,5-ICAN, while in 2,6-ICAN this requires changes in the geometry as well. Therefore, in 2,6-ICAN, a charge transfer does take place, but in a non-emissive path via excited state relaxation.

In order to learn more about the ground state electronic properties of the 1,4- and 2,6-ICAN isomers, they were dissolved in various solvents, and their UV-Vis spectra were recorded in the wavelength range of 200 nm to 700 nm. The solvents were chosen to cover a broad range of solvent polarity, spanning from the non-polar hexane to the polar DMSO. The UV-Vis spectra of 1,4-ICAN and 2,6-ICAN along with 1,5- ICAN in different solvents.

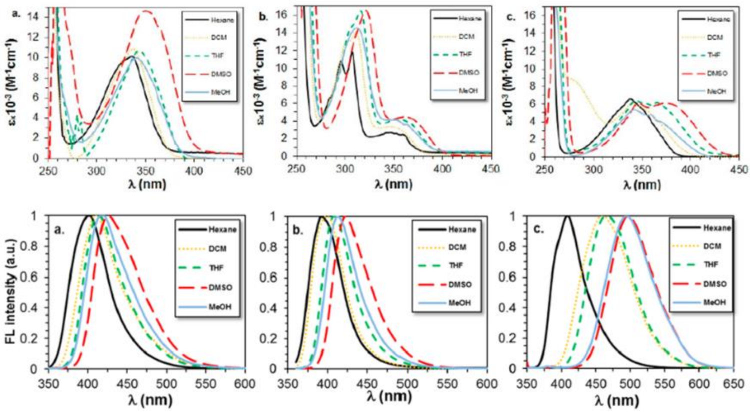

Figure 13.: UV-vis absorption (top) and normalized emission (bottom) spectra of 1,4-ICAN (a), 2,6-ICAN (b) and 1,5-ICAN (c) isomers recorded in different solvents.

The normalized fluorescence emission spectra for the 1,4-ICAN and 2,6-ICAN isomers are shown in Figure 13 (bottom). The fluorescence emission spectra of both 1,4-ICAN and 2,6-ICAN in various solvents show unstructured, single bands, similar to those of 1,5-ICAN, revealing positive solvatochromic shifts upon increasing the solvent polarity from n-hexane to DMSO.

In order to quantify the solvatochromic effect induced by the solvents of different polarity, the fluorescence emission maxima (ν_{Em}) are plotted as a function of the empirical solvent polarity parameter ET(30) and the Lippert–Mataga (LM) plot, i.e., a plot for the Stokes shifts ($\Delta\nu_{SS}$) versus the orientation polarizations (Δf_{LM}) are constructed and presented in Figure 14.

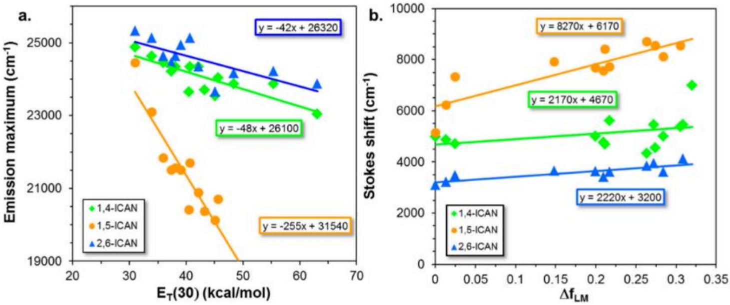

Figure 14.: Variation of the fluorescence emission maximum with the empirical solvent polarity parameter ET(30) (a), and the Lippert–Mataga (LM) (b), plots for the 1,4-ICAN, 1,5-ICAN, and the 2,6-ICAN isomers.

As it turns out from Figure 14 a, there are linear correlations between the fluorescence emission maximum and ET(30) for all the three fluorophores. On the other hand, as judging from the corresponding slopes illustrated in Figure 4a, it is also evident that the highest solvatochromic shift, i.e., the highest slope can be observed for the 1,5-ICAN isomer (255 $kcal^{-1}cm^{-1}mol$), while these values are very similar for the 1,4-ICAN (48 $kcal^{-1}cm^{-1}mol$) and the 2,6-ICAN (42 $cal^{-1}cm^{-1}mol$) isomers. To gain quantitative insight, the Lippert–Mataga equation can be employed.

According to Figure 14 b, the 1,5-ICAN isomer yields much higher slope (8270 cm^{-1}) obtained from the LM-plot than do the 1,4-ICAN and 2,6-ICAN isomers, whose LM-plots give very similar slopes (2170 and 2220 cm^{-1}, respectively). In addition, the Stokes shifts at $\Delta f_{LM} = 0$, i.e., the intercepts of the lines determined from the LM-plots decrease in the order of 1,5-ICAN > 1,4-ICAN > 2,6-ICAN. The LM-plot can also be used to estimate the difference in the values of the excited and the ground state dipole moments, i.e., $\Delta\mu = \mu_E - \mu_G$.

3. List of publications related to the dissertation

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Subject: PhD Publication List

Candidate: Sándor Lajos Kovács
Doctoral School: Doctoral School of Chemistry
MTMT ID: 10068123

List of publications related to the dissertation

Foreign language scientific articles in international journals (4)

1. Nagy, M., Rácz, D., Nagy, Z. L., Fehér, P. P., **Kovács, S. L.**, Bankó, C., Bacsó, Z., Kiss, A., Zsuga, M., Kéki, S.: Amino-isocyanoacridines: Novel, Tunable Solvatochromic Fluorophores as Physiological pH Probes.
   Sci. Rep. 9, 1-39, 2019. ISSN: 2045-2322.
   IF: 3.998

2. **Kovács, S. L.**, Nagy, M., Fehér, P. P., Zsuga, M., Kéki, S.: Effect of the Substitution Position on the Electronic and Solvatochromic Properties of Isocyanoaminonaphthalene (ICAN) Fluorophores.
   Molecules. 24 (13), 1-15, 2019. ISSN: 1420-3049.
   DOI: http://dx.doi.org/10.3390/molecules24132434
   IF: 3.267

3. Nagy, M., **Kovács, S. L.**, Nagy, T., Rácz, D., Zsuga, M., Kéki, S.: Isocyanonaphthalenes as extremely low molecular weight, selective, ratiometric fluorescent probes for Mercury(II).
   Talanta. 201, 165-173, 2019. ISSN: 0039-9140.
   DOI: http://dx.doi.org/10.1016/j.talanta.2019.04.007
   IF: 5.339

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4. Nagy, M., Rácz, D., **Kovács, S. L.**, Lázár, L., Fehér, P. P., Purgel, M., Zsuga, M., Kéki, S.: New blue light-emitting isocyanobiphenyl based fluorophores: Their solvatochromic and biolabeling properties.
   J. Photochem. Photobiol. A-Chem. 318, 124-134, 2016. ISSN: 1010-6030.
   DOI: http://dx.doi.org/10.1016/j.jphotochem.2015.12.006
   IF: 2.625

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Total IF of journals (publications related to the dissertation): 15,229

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