Ph.D. thesis

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Electronic spectroscopic study of signalling molecules

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Introduction

The Ph.D. thesis is about the electron spectroscopic investigation of molecules that play the role of signalling agents in certain processes. The applied electronic spectroscopic methods were complemented in each case by theoretical calculations.

The design, synthesis and spectroscopic investigation of sensor molecules which incorporate the chiral recognition- and also the optical signalling function is nowadays one of the major trends in chemical research. These molecules can be applied in analytical systems based on enantioselective complex forming and detection of optical phenomena (such as UV-visible absorption, fluorescence quenching etc.).

In the first part of the dissertation the structural and complex forming properties of two such host molecules are studied by steady state optical spectroscopic methods concerning their possible application in optical sensors.

Fluorescence probes are molecules which respond to the change of various environmental parameters (e.g. viscosity, polarity, presence of water molecules) by the drastic change of their fluorescence. Bound to the peptide or protein backbone these molecules can provide valuable information about the structural, conformational and functional changes of biomolecules induced by environmental effects. The goal of the thesis’ second chapter was to unravel the mechanism of the fluorescence quenching of four indoline derivatives. For this purpose various steady state, time-resolved and laser techniques were used. The understanding of the quenching dynamics can promote the application of these molecules as fluorescence probes.

The asymmetric epoxidation of chalcone, leading to \textit{trans}-chalcone epoxide is frequently used for the characterisation of different chiral phase-transfer catalysts. The absolute configuration and the enantiomeric excess of the reaction product is indicative of the manner and the effectiveness of the chiral induction resulted by the catalyst. \textit{Trans}-chalcone epoxides are also of great importance as intermediates of natural products and pharmaceuticals. In the third chapter the CD spectroscopic determination of the absolute configuration of \textit{trans}-chalcone epoxides is presented. Also the complete interpretation of the CD spectra was carried out with the help of quantum chemical calculations.

Materials, experimental methods

At the Department of Organic Chemistry and Technology of BUTE there is a tradition for the synthesis and design of supramolecular sensor molecules. Also the asymmetric synthesis is an intensively studied field there, particularly the asymmetric epoxidation of the double bond. To raise the performance and the selectivity of the latter reaction, different phase-transfer catalysts containing monosaccharide and crown ether subunits are being tested. The calixcrown host molecule, studied in the first chapter together with the indoline derivatives were synthesized in the research group of Dr. István Bitter. The \textit{trans}-chalcone epoxides of the third chapter were prepared by the research group of Dr. Péter Bakó. Finally the cyclohexane based fluorescent host molecule was prepared at the Organic Chemistry Department of the University of Valencia, in the laboratory of Dr. Ana Maria Costero.
Figure 1.: Chiral host molecules studied in the first chapter of the thesis: a) The (S) isomer of the chiral, chromogene calix[4]crown-6-ether /IUPAC name: 5,17-(2,4-dinitrophenylazo)-25,27-calix[4](2-1,1’-bi-2-nafto-crown-6)arene/ [(S)-CX]. b) cyclohexane based, fluorescent host /IUPAC name: (diethyl 4,5-bis(3-naphtalene-1-il) thioureido) cyclohexane-1,2-dicarboxilate) [CHG].

In the first chapter, the complex forming reaction of the CX host molecule (Fig.1.a) with organic chiral amines was investigated with the help of steady state UV-visible absorption-, fluorescence and CD-spectroscopic measurements. To modelise the geometry of the host molecular mechanical and semi-empirical calculations were implemented.

The absolute configuration of CHG molecule and trans-chalcone epoxides were determined by CD spectroscopic measurements. To determine the geometry of the dominant conformer (or conformers) and to assign the experimental CD bands, molecular mechanical and semi-empirical calculations were implemented in case of the large and flexible CHG, and further DFT calculations in case of the smaller and rigid trans-chalcone epoxides.

The fluorescence quenching mechanism of indoline derivatives in protic solvents was explored by time-correlated single photon counting, flash photolysis (with the detection of the transient absorption) and steady state fluorescence spectroscopy. To support the experimental results, ab initio quantum chemical calculations were implemented on the ground and excited states of the hydrogen-bonded complexes between protic solvents and indoline-derivatives.
New results

1.A. Spectroscopic study of the complex forming reaction between a chiral, chromogenic calix[4]crown-6-ether and organic chiral amines

A binaphthyl-appended calix[4]crown-6-ether with two 2,4-dinitrophenylazo chromophore units (Fig. 1.a) was tested as chiral host in its reactions with the enantiomers of α-methylbenzilamine and phenylglycinol [PGO] in acetone and acetonitrile. The visible absorption spectra indicate a two step process, first a nonenantioselective proton transfer from the host to the guest, followed by complex formation, which proved to be enantioselective (Fig. 2.). The UV fluorescence of the host arising form the binaphthyl moiety is quenched by K⁺-ions but not by the amine guests, showing that the interaction between the binaphthyl group and the complexed amines is weak. In the UV range of the CD spectra an intensive negative/positive exciton couplet appears, arising form the coupling of the 2^1B_3u transitions of the naphthalene units of the R/S isomer of the host. From the sign and other parameters (namely, the true splitting and amplitude) of this couplet, the absolute configuration of the host and the torsion angle between the naphthalene planes can be deduced. In the visible range a positive/negative Cotton-effect belongs to the phenolic form of the R/S enantiomers of the host. The addition of the guest is accompanied by the appearance of a weak positive-negative/negative-positive asymmetric band-pair in case of R/S enantiomers of the host, irrespective of the chirality of the guest. These bands can be assigned to the deprotonated and the complex forms of the host.

Figure 2.: a) Absorption spectra: Acetonitrile solution of (S)-CX host, with the addition of 100 and 200 equivalents of (R-) and (S)-PGO. b) Acetonitrile solutions of (S)-CX [5·10⁻⁵] with 200 equivalents of (R-) and (S)-PGO.
1.B. CD spectroscopic study of a chiral, cyclohexane based fluorescent chemosensor molecule

The determination of the absolute configuration of CHG (a cyclohexane based fluorescent host, capable for the discrimination of chiral dicarboxilate enantiomers) was carried out with the help of CD spectroscopic measurements and quantum chemical calculations. The host molecule is quite flexible, for the determination of the geometry of the dominant conformers, molecular mechanical and semi-empirical calculations were performed. The conformational analysis resulted in 29 conformers. The energy of 24 of them differed by less, than 3 kcal/mol from the energy of the most stable conformer. These 25 (most stable + 24) conformers were taken into account henceforth. (At room temperature they cover 99.4% of all the molecules).

In the CD spectrum of the host with positive optical rotation, an intensive exciton couplet appears in the absorption range of the naphthyl-thiourea groups (Fig. 3.a). Based on ZINDO/S level calculations the appearance of the couplet is the consequence of the interaction between the $2^1B_{3u}$ symmetry excited states of the naphthalene rings. The absolute configuration of the host was determined by the exciton chirality rule, which in case of $(1S,2S,4S,5S)$ absolute configuration predicts a negative exciton couplet for all 25 conformers, based on the torsion angle of the electronic transition dipole moments of the coupling excited states (Fig. 3.b). Thus, the absolute configuration of (+)-CHG, which gives a negative couplet, is $(1S,2S,4S,5S)$. 
2. Fluorescence decay dynamics of cyano-methyl-indoline and its analogons

In the second chapter of the dissertation the photophysical and photochemical properties of indoline and three of its derivatives (Fig. 4.) were studied in H-donating solvents and solvent mixtures of varying polarity.
Figure 4.: Structures of the studied indoline derivatives.

Fluorescence lifetimes of the four compounds in n-hexane, acetonitrile and ethanol were obtained by time correlated single photon counting. Fluorescence quenching of cyano-substituted molecules were observed in ethanol. The fluorescence and triplet quantum yields of the molecules were measured by steady state fluorescence spectroscopy and transient absorption techniques in the same solvents. From these data, the internal conversion quantum yields and the rate constants for the standard deactivation processes were calculated. (Figure 5. shows the comparison of the rate constants of the standard deactivation processes).

Figure 5.: The comparison of the rate constants for the standard photophysical deactivation processes.

Experimental results in various solvents and solvent-mixtures

The rate constant of the internal conversion of the cyano-substituted derivatives increased by one order of magnitude in ethanol as compared to solutions in n-hexane or acetonitrile. From the comparison of the photophysical parameters, it can be concluded that the N-methylation does not have an influence on the photophysical properties (or the quenching mechanism).
Taken into consideration the fluorescence lifetimes obtained in solvents with varying polarity and proton-donating ability (propylene-carbonate, acetonitrile, tert-butanol, 2-propanol, methanol, ethylene-glycol) it can be established that the quenching is relevant only in solvents that contain OH group or can take part in a hydrogen-bonded interaction by providing the proton.

Also the fluorescence of the non-cyanated compounds is quenched in water (the fluorescence lifetime is reduced by one order of magnitude with respect to the organic solvents) The fluorescence lifetime of the cyanated compounds is reduced by two orders of magnitude as compared to the results in organic solvents.

Measurement of the fluorescence lifetime at different temperatures was carried out in neat solvents, for the determination of the activation energies of the different quenching processes. The slopes of the respective Arrhenius plots yield activation energies 33 and 32 kJ/mol for MI and I in water. The value of the activation energy is much less for the cyanated compounds, only 10 and 12 kJ/mol for CI and CMI in ethanol. The difference indicates the diverse nature of the quenching processes that take place in case of the cyanated and the non-cyanated compounds.

The results of the fluorescence lifetime measurements in ethanol-water, ethanol-acetonitrile and water-acetonitrile mixture solvents (at different compositions) strengthen the similarity of the quenching processes of CI and CMI and the dissimilarity of the mechanisms explaining the quenching of MI and the cyanated compounds. The results also suggested that the same quenching process takes place in ethanol and water in case of the cyanated compounds.

The Stern-Volmer representation of the data implies the dynamic nature of the quenching process in case of the cyanated compounds in ethanol and water.

The quantum yields of the hydrated electron formation were obtained by transient absorption measurements. The photoionization is relevant in case of the non-cyanated compounds, but negligible for the cyanated ones.

Quantum chemical calculations, deactivation mechanisms.

Based on the previously discussed observations two solvent induced quenching mechanisms were presumed.

In case of the cyano-substituted derivatives, the quenching that occurs in water and ethanol can be explained by the formation of a CN-HO hydrogen bond between the solute and the solvent. The other mechanism refers to the quenching of the non-cyanated compounds by water molecules and is connected to the formation of hydrated electrons (photoionization of the solute).

Quantum chemical calculations were performed on the ground and excited states of the hydrogen-bonded complexes between protic solvents (water and ethanol) and MI as well as CMI. Hydrogen-bonded configurations involving the CN substituent and a solvent OH group are stable both in the ground and the first excited singlet states, whereas the corresponding complex at the ring amino nitrogen is stable in the ground state only (Figure 6.).
Based on theoretical calculations, the formation of the complex results in new, low frequency normal modes belonging to the CN-HO bond (at 20-100 cm⁻¹), thus the density of the vibronic states around the S₁ state increases, consequently the probability of the internal conversion grows. The generation of an excited state collision complex stabilized by the CN-HO bond is a prime candidate for the explanation of the observed quenching by the first mechanism.

No stable hydrogen-bonded complex was found for the excited MI molecule, which excludes the possibility of quenching as a result of hydrogen bonding. The first excited state of MI is a Rydberg-like state. The vertical ionization potential of this state calculated at the CIS/6-31++G** level is only 0.5 eV. The diffuse nature of the S₁ state apparently facilitates the ionization of the molecule, hence supporting the electron ejection mechanism proposed on the basis of the experimental results.

3. Determination of the absolute configuration of the reaction products of asymmetric epoxidation

The absolute configuration of several trans-chalcone epoxides – unsubstituted and having different ortho- and para- substituents on their aromatic rings – were established by CD spectroscopy, based on the sign of their carbonyl n→π* transition, applying the octant rule. For the application of the rule, the geometries of the dominant conformers are required. To obtain the conformers, conformational analysis was carried out. This was followed by a DFT/6-31++G** level geometry optimization in order to have the geometries. The results of the conformational analysis (and a further RI-MP2/TZPP calculation) suggested two conformers for the unsubstituted compound. The energy of the most stable one is 6 kJ/mol lower than the energy of the other. That means that 92% of the molecules are present in the most stable conformation at room temperature. If the absolute configuration is (2R,3S), the octant rule predicts a negative
carbonyl sign. (The octant projection diagram of most stable conformation of the (2R,3S)-unsubstituted trans-chalcone epoxide can be seen in Fig. 7.a).

![Diagram of octant projection](image)

Figure 7.: a) The octant projection diagram of the (2R,3S) isomer. b) The experimental CD spectrum of the unsubstituted (–)-trans-chalcone epoxide and the calculated CD spectrum of the (2R,3S)-unsubstituted trans-chalcone epoxide (obtained from TD-DFT calculations).

The octant rule predicts a negative carbonyl band for all the investigated derivatives if (2R,3S) absolute configuration is assumed. The chalcone epoxides with (–)-optical rotation uniformly gave a negative carbonyl sign, thus, the corresponding absolute configuration is (2R,3S). (In opposite case: Each (+)-compound gave a positive carbonyl sign; the absolute configuration of these compounds is (2S,3R)). The spectra have been assigned with help of the energies, oscillatory strengths, and rotatory strengths of the singlet transitions obtained from DFT calculations. The TD-DFT calculations – which predicted band signs agreeing well with the experimental ones (Fig. 7.b) – did not confirm the applicability of the exciton chirality rule on the CD bands present in the lower wavelength range of the spectrum.
Thesis points

1.A/1. Based on absorption measurements, the complex forming reaction between the \textbf{CX} host and the chiral guest amines is a two-step process: first a nonstereoselective proton transfer takes place, which is followed by the complex forming [1, 2].

1.A/2. The complex forming reaction between the \textbf{CX} host molecule and the enantiomers of phenylglycinol shows enantioselectivity in acetonitrile [2].

1.A/3. The UV fluorescence of the host arising from the binaphthyl moiety is not quenched by the amine guests, and the addition of the guest does not change the CD signals of the binaphthyl group. These observations imply that the interaction between the binaphthyl group and the complexed amines are weak and cannot be demonstrated directly by the experiments [2].

1.B/1. The exciton couplet appearing in the CD spectrum of the (+)-\textbf{CHG} compound is the consequence of the coupling between the excited states of the transitions belonging to the naphthyl-thiourea groups. The coupling excited states can be assigned to the $2^1\text{B}_3u$ transition of the naphthalene, (for which the electronic transition dipole moment vector is parallel to the long axis of the naphthalene ring) [7].

1.B/2. In case of the 25 most stable conformers of the (1$S$,2$S$,4$S$,5$S$)-\textbf{CHG} compound (obtained from MM and AM1 calculations) the electronic transition moments of the $2^1\text{B}_3u$ symmetry transition of the naphthalene rings show a negative torsion angle, thus a negative exciton couplet belongs to the (1$S$,2$S$,4$S$,5$S$)-absolute configuration [7].

2/1. In case of the cyano-substituted indoline derivatives the strong decrease of the fluorescence lifetime, along with the increase of the internal conversion rate constant is observed in solvents containing an OH group (water, ethanol), compared to n-hexane and acetonitrile. The observed quenching can be explained by the formation of an excited state collision complex which is stabilized by a hydrogen-bond between the cyano group of the solute and the OH group of the solvent. (The observed process leads to the very effective deactivation of the cyanated compounds in water) [6].

2/2. The fluorescence lifetime of the non-cyanated compounds is reduced by one order of magnitude in water, as the consequence of the formation of hydrated electrons [6].

2/3. The \textit{N}-methylation has no major effect on the photophysics and photochemistry of the investigated compounds. [6].

3/1. The absolute configuration of optically active \textit{trans}-chalcone epoxides – yielded by the asymmetric epoxidation of the chalcone – can be determined from their CD spectrum based on the sign of their carbonyl $n\rightarrow\pi^*$ transition at 330 nm, applying the octant rule. The \textit{ortho}- and \textit{para}-substituents on the aromatic rings do not change the sign of the carbonyl band [3, 4, 5].
The octant rule predicts a negative carbonyl band for the (2R,3S) absolute configuration, and positive carbonyl band for the (2S,3R) configuration [3, 4].

TD-DFT and RI-CC2 calculations implemented on the (2R,3S)-unsubstituted trans-chalcone epoxide indicate a negative carbonyl sign. All the band signs of the calculated CD spectrum of the (2R,3S)-unsubstituted trans-chalcone epoxide (obtained from RI-CC2 calculations) are identical to the CD band signs of the experimental spectrum of the unsubstituted trans-chalcone epoxide with negative optical rotation [5].

**Possible applications**

The results of the first chapter might be utilized at the design of host molecules applicable in optical sensors for the recognition of physiologically important molecules.

The results of the second chapter can well be applied in the design of water-sensitive fluorescence probes for biological systems.

Based on the results of the third chapter, the absolute configuration of trans-chalcone epoxides can be determined routinely. This can fasten the evaluation of the synthetic work. Finally the results demonstrate the performance and the reliability of modern quantum chemical methods with respect to the calculation of the CD spectrum.
List of publications and presentations

Publications that serve as the basis of the dissertation:


Publications not connected to the dissertation:

List of conference presentations and posters:


**Poster not connected to the dissertation:**