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On the Inclusion Ability of a Fluorinated Fluorescent Indolizine β–Cyclodextrin Sensor towards Volatile Organic Compounds

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On the Inclusion Ability of a Fluorinated Fluorescent Indolizine β–Cyclodextrin Sensor towards Volatile Organic Compounds [#]

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Abstract

A fluorine fluorescent indolizine β–cyclodextrin sensor has been studied on an experimental and theoretical point of view for its sensing ability towards benzene and toluene. The ability of the sensor to behave as a fluorescent probe has been evaluated, as well as the formation constant values (measured using a spectral displacement method associated to a dedicated algorithmic treatment). The sensor conformation has been investigated by molecular modeling, and it seems that the open structures of the sensor are the most probable one in aqueous medium. According to the docking study realized for each guest, it appears that the open conformations represent the more stable complexes. Moreover, the experimental results are consistent with the computed complexation energies.

Keywords. Molecular docking; indolizine; β–cyclodextrin; β–cyclodextrin sensor; volatile organic compounds; VOCs; fluorescence detection; formation constant.

1 INTRODUCTION

Cyclodextrins are cyclic oligomers of D–glucopyranose, which can accommodate in their cavities a large variety of organic compounds. In addition, their negligible cytotoxic effects are an important attribute in applications such as drug carrier, food and flavours, cosmetics, packing, textiles, separation processes, environment protection, fermentation and catalysis [1].

Besides, the modification of cyclodextrins with chromophore fragments has aroused interest because it transforms such an optically inert macrocycle into an active one [2]. In particular,

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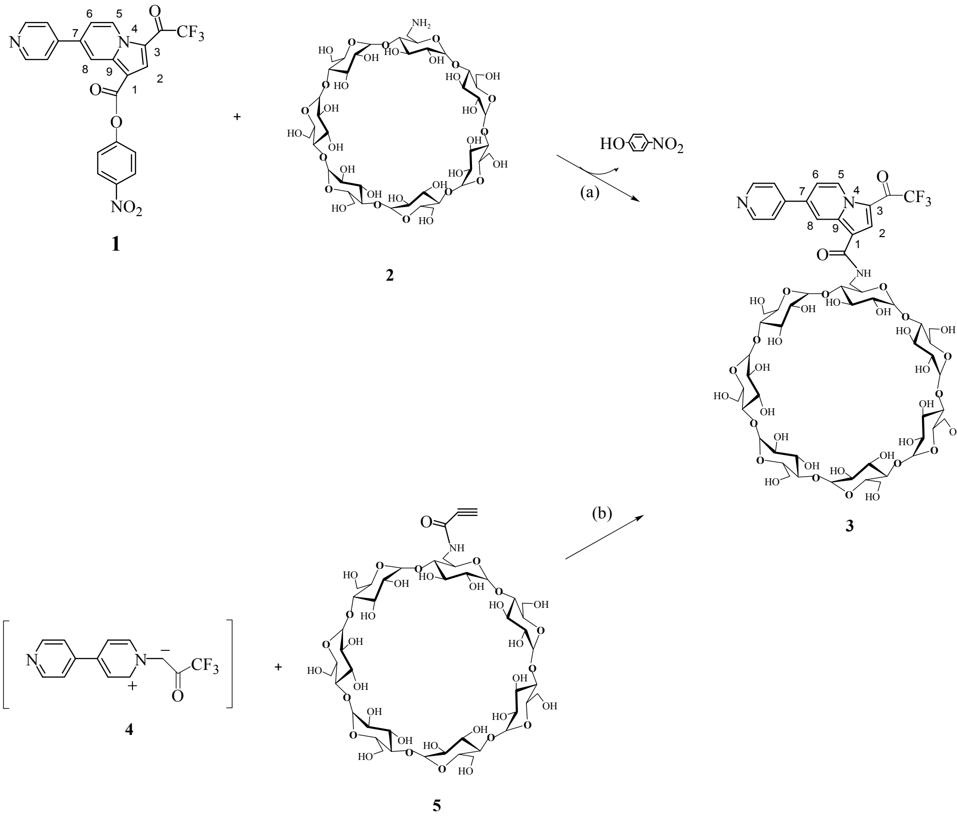
fluorescent cyclodextrins may exhibit fluorescent sensing abilities for organic guest [3]. Within this scope, we recently reported the fluorescent host–guest systems using β–cyclodextrin modified with a indolizine fluorescent moiety [4]. The synthetic procedure of the fluorine fluorescent indolizinic β–cyclodextrin (**3**) together with its sensing behavior towards adamantanol (Scheme 1) has been described in a previous paper [5].



Scheme 1. Synthesis of the fluorescent indolizine β–cyclodextrin sensors.

Two different synthetic ways have been employed (Scheme 1). The first one (a) involves an amidation of 6–deoxy–6–amino–β–cyclodextrin **2** and an esteric indolizine derivative **1**. According to the second way (b), the same fluorine fluorescent sensor **3** have been obtained by a 3+2 cycloaddition reaction between ylide **4**, generated “in situ” from its corresponding salt, and 6–propynamido β–cyclodextrin **5**.

The aim of this experimental and theoretical study is to probe the ability of the compound **3** to behave as chemical sensor for volatile organic compounds (VOCs). In our study, benzene and toluene were chosen as guest. On an experimental point of view, the determination of the formation constant has been realized by means of a spectral displacement method (UV–visible spectroscopy), while the sensitivity factor was investigated by fluorescence spectroscopy. Molecular modeling by MM3 and AM1 methods was then used to compute the inclusion energies and to obtain structural information on the inclusion complexes.

2 MATERIALS AND METHODS

2.1 Visible Spectra

Spectra were recorded at 293K using a Perkin Elmer Lambda 2S double beam spectrometer and a quartz cell with optical path length of 1.00 cm. All compounds were dissolved in phosphate buffer at pH 5.8. For the spectral displacement method, the sensor was dissolved in a methyl orange (MO) solution and each compound, benzene and toluene, was dissolved in the resulting solution in order to avoid spectral variation due to inconstant concentration of the absorbing species.

2.2 Formation Constant Determination

Evaluation of sensor inclusion capacity towards benzene and toluene has been carried out by use of UV–Visible spectroscopy.

2.2.1 Direct titration method

First, the sensor/MO system is characterized by a direct titration method. For a 1:1 molar ratio complex the calculation of formation constant K_f was developed as follows:

$$\text{MO + SENSOR} \rightleftharpoons \text{MO / SENSOR}$$

$$K_f = [\text{MO / SENSOR}] / [\text{MO}]\,[\text{SENSOR}] \qquad (1)$$

$$K_f = [\text{MO / SENSOR}] / (\,[\text{MO}]_T - [\text{MO / SENSOR}]\,)*(\,[\text{SENSOR}]_T - [\text{MO / SENSOR}]\,) \qquad (2)$$

$$[\text{MO / SENSOR}] = -\frac{1}{2}\sqrt{\left[(\frac{1}{K_f} + [\text{SENSOR}]_T + [\text{MO}]_T)^2 - 4[\text{SENSOR}]_T[\text{MO}]_T\right]} + \frac{1}{2}(\frac{1}{K_f} + [\text{SENSOR}]_T + [\text{MO}]_T) \qquad (3)$$

where K_f and T stand for formation constant and total respectively. For a given value of K_f, [MO/SENSOR] is known and the spectral characteristic of the complex can be calculated. The algorithm treatment was then applied to minimize the difference of the spectral characteristic over the various solutions. The calculations were realized with the first derivatives of UV spectra in order to avoid any spectral influence diffraction phenomena [6].

2.2.2 Spectral displacement method

The association constants of benzene and toluene with the sensor were determined by applying a spectral displacement method with MO in its basic form. Two 1:1 equilibriums may take place between SENSOR, MO and Guest (G):

$$\text{MO + SENSOR + G} \rightleftharpoons \text{MO / SENSOR + G} \rightleftharpoons \text{SENSOR / G + MO}$$

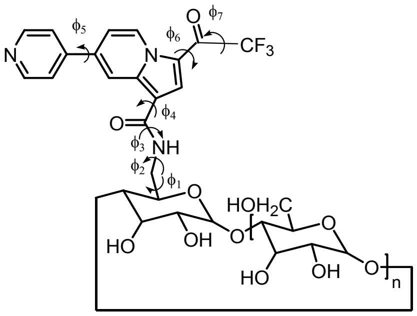
While concentrations of MO and SENSOR are kept constant, the addition of G implies an absorbance increase, proportional to the expulsion of MO from the SENSOR cavity. The formation constant of the SENSOR/G complex can therefore be deduced from this absorbance difference. An algorithmic method was used for the data treatment. Its principle consists in the calculation of the concentration of the complexes by considering the two equilibriums successively in an iterative way [6,7]. Spectra were recorded between 520–530nm for a MO concentration fixed at 0.1mM. This wavelength range corresponds to the optimal spectral variation between the free and complexed forms of MO.

2.3 Fluorescent Measurements

The measurements were carried out with a Perkin Elmer LS–50B fluorimeter at 293K. The excitation wavelength of the fluorescence spectrum was 370 nm and excitation and emission slits were 4 nm.

2.4 Molecular Modeling

The sensor molecule was built starting from the data provided by the Structural Data Base System of the Cambridge Crystallographic Data Center. The calculations were made using the CAChe Library [8] on a PC–Computer. To obtain the most stable conformers of sensor **3** we used a general procedure of MM3 multiconformational search described in various papers [9]. This search consists in studying the ΔE, potential energy variation according to the variation of the various dihedral angles by rotational increments of 15°. The minimal values of ΔE are chosen according to the curve scribing. The various dihedral angles defining the sensor conformations are described in Scheme 2.



Scheme 2. Global structure of the sensor.

The rotations corresponding to dihedral angle φ_5, φ_6 and φ_7 are not involved directly on the positioning of fluorescent fragment in respect to primary face of β–CD fragment. The torsions according φ_1 and φ_2 present a high proximity with the toroidal cycle of β–CD and consequently a reduced freedom. Thus, we may conclude that the two rotations described by φ_3 and φ_4 are sufficient to find the more stable conformations in the conformational search. As a consequence, only φ_3 and φ_4 are explicitly varied in the conformational search, while φ_1, φ_2 , φ_5, φ_6 and φ_7 are only energy minimized. Once the minima have been obtained by this MM3 search, each conformation is freely minimized according to AM1 hamiltonian in gas phase and aqueous medium (COSMO solvent field). Indeed, the energy obtained for AM1 in water should be the most suitable parameter to describe the sensor conformations in aqueous medium.

If AM1 Hamiltonian has been preferred for the determination of the sensor intramolecular structure, the docking of guests (benzene and toluene) in respect to β–CD inner cavity has been performed on the basis of MM3 force field. The intermolecular interactions are easily reproduced by molecular mechanics, especially when charge transfers are not involved, and this is the case for cyclodextrin complexes. The docking has been realized using a dummy atom, centrally placed in the cyclodextrin cavity. The guest in then pulled towards the cyclodextrin host and MM3 energy is monitored with a constant length step, the distance between the dummy atom and the guest being imposed as a constraint. Finally, for all most stable conformers of inclusion compounds, all constraints are removed and a new geometry optimization is made. Since the inclusion of benzene may occur with the methyl group or the phenyl group first, we envisaged these two regioselectivities. For each complex, the theoretical parameter which has been calculated to evaluate the inclusion capacity of sensor **3** is the computed stabilization energy (ΔE, kcal/mol), defined as the difference between total energy of the inclusion complex and the sum of energies for individual host and guest.

3 RESULTS AND DISCUSSION

3.1 Experimental Study

The visible spectra used in direct titration were recorded between 520–530 nm for a concentration of MO of 0.1mM and a variation of sensor concentration of 0.1mM to 0.0167mM. The obtained data are in agreement with a complex ratio SENSOR/MO 1:1 and leads to an association constant of 8339 M^{-1} ±10 %. For benzene and toluene, the quantitative data are obtained from spectral displacement method by addition of the guest (4.10mM of benzene and 4.13mM of toluene) on the SENSOR / MO solution (Figure 1) and by using an algorithmic procedure [6,7]. For comparison, the similar formation constants involving the β–CD (estimated by the same experimental procedure) are also given in Table 1.

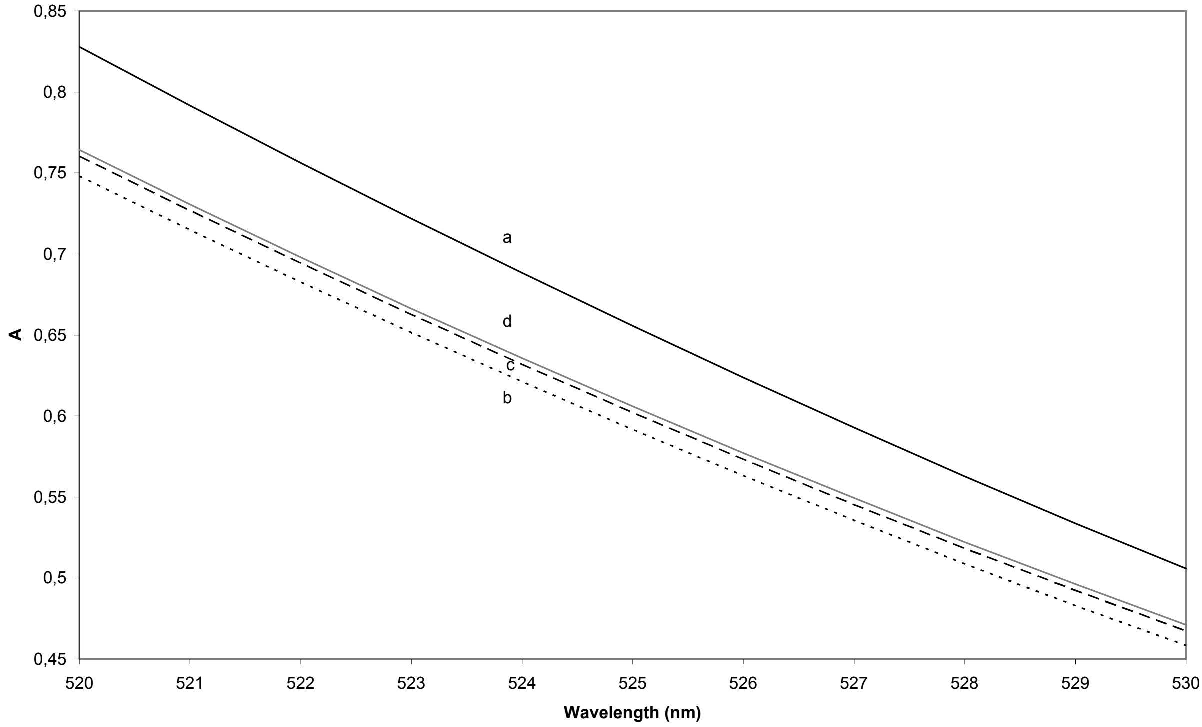


Figure 1. Absorption spectra for solutions containing (a) methyl orange 0.1mM, (b) methyl orange 0.1mM and sensor 0.1mM, (c) methyl orange 0.1mM and sensor 0.1mM and benzene 4.10mM, (d) methyl orange 0.1mM and sensor 0.1mM and toluene 4.13mM.

Table 1. Formation Constants (M^{-1})

Guest	SENSOR	β–CD
MO	8339	2500
Benzene	46	82
Toluene	61	102

The obtained values show that the toluene inclusion compound is stronger than the benzene one, whatever cyclodextrin is concerned, even if the differences are very weak. Moreover, even if the determination of small formation constant is subject to experimental error, it seems that the guest binding ability of the sensor is not enhanced by the existence of the hydrophobic cap. But it has to be mentioned that this is not an essential point to present a sensing ability for benzene and toluene.

The addition of benzene and toluene to a solution of sensor leads both to an increase of the fluorescence emission (Figure 2). The fluorescence intensity of the modified cyclodextrin is affected by the presence of the guest molecule, but as can be seen in Figure 2, saturation appears for a concentration of toluene about 6mM. For higher concentrations, the response of the sensor is not concentration dependant.

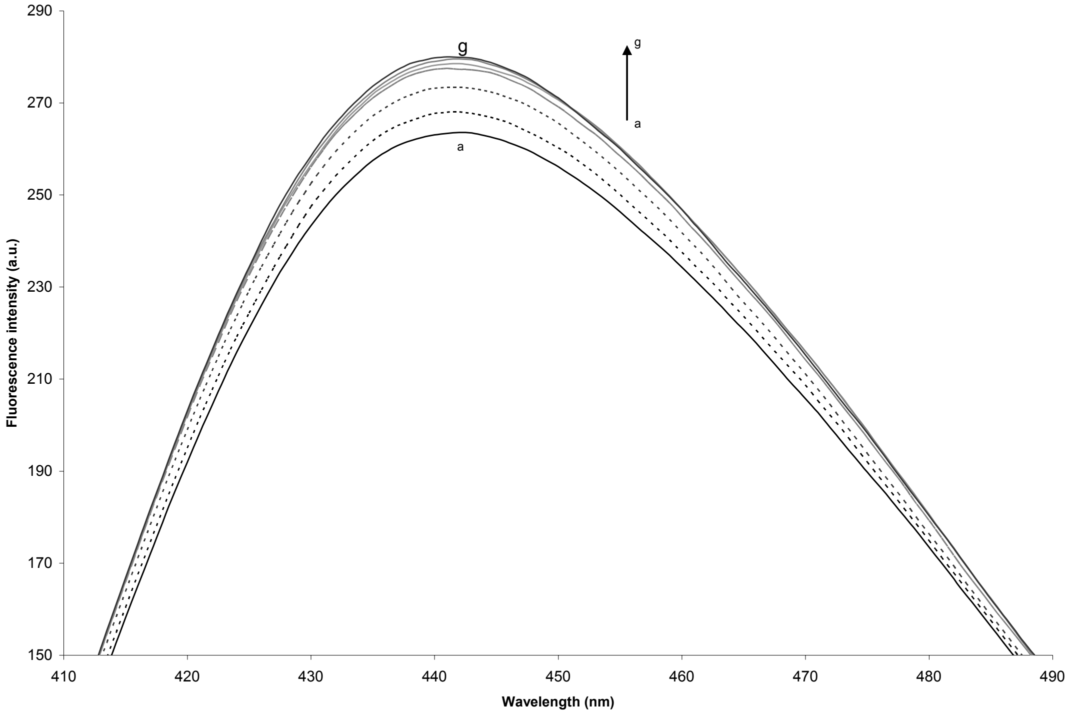


Figure 2. Fluorescence spectra of the sensor in aqueous solution (a) (0.1mM, 25°C), at various concentrations of toluene (b) 1.9mM, (c) 3.8mM, (d) 5.7mM, (e) 7.6mM, (f) 9.5mM, (g) 19mM.

To calculate the sensing abilities of the sensor, the $\Delta I/I_0$ value was used as the sensitivity factor. Here ΔI is $I–I_0$, where I_0 is the fluorescent intensity of the host alone at 439 nm, and I corresponds to the mixture of host and guest [1,b].

Table 2. Sensitivity Factor for Benzene and Toluene

Guest	$\Delta I/I_0$
Benzene	0.099
Toluene	0.053

As a consequence of the low variation of intensity, the sensitivity factors are not high for the two guests, if compared to previously synthesized fluorescent indolizine β–cyclodextrin sensor [10], even if the formation constants of these two sensors with our guests are in the same order of magnitude.

3.2 Theoretical Study

A MM3 multiconformational search has been performed for the sensor **3**, on the basis of φ_3 and φ_4 dihedrals. These are the key dihedrals that control the relative position between the fluorescent and cavity moieties.

Each structure obtained by the MM3 search has then been minimized without imposing any restrictions, on the basis of AM1 hamiltonian in aqueous and gas phase. Our search thus reveals the existence of six principle conformers named S_1 to S_6, S_1 being the most stable structure and S_6 the less stable one, as defined by AM1 in water.

Two types of sensor structures may be identified: one with an open cavity (S_1 and S_2) and another with a capped cavity (S_3, S_4, S_5 and S_6). Among the six conformers, if the ground state calculations recommend the conformer S_6 as most stable, the water solvent calculation indicates the conformer S_1 as the most probable (Table 3, Figure 3). This result was predictable since S_1 structure presents the wider solvent surface accessible area, in opposition to capped structures that are less exposed to water.

Since our experimental study takes place in aqueous medium, we may consider that the conformer S_1 is the key structure involved in the observed complexation. The fact that at least 3 kcal/mol are required to convert S_1 to any other conformers leads to think that S_1 may be the predominant species in water, and thus that S_1 affords the greatest contribution to the affinity of the studied host–guest inclusion compounds.

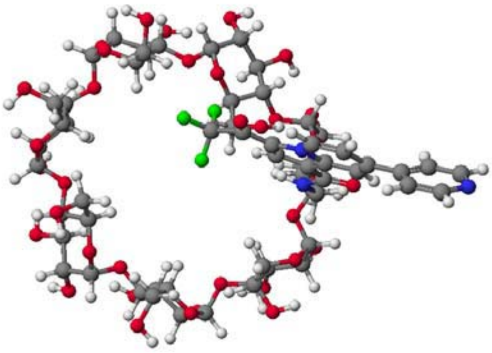
Table 3. Formation Enthalpy for the Most Stable Conformers of the Sensor **3**

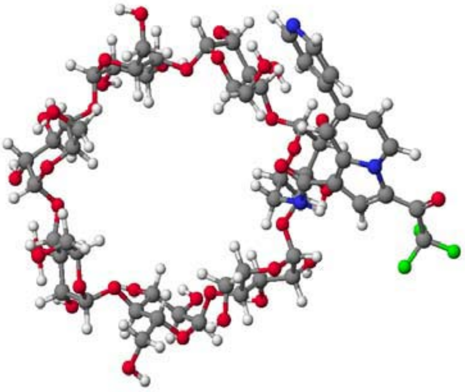
	ΔH_s kcal/mol (H_2O), AM1	ΔH kcal/mol (gas), AM1
S_1	–56.36	–1706.3339
S_2	–53.22	–1712.0146
S_3	–53.01	–1714.1434
S_4	–50.89	–1715.3390
S_5	–50.76	–1709.2178
S_6	–50.08	–1716.8983

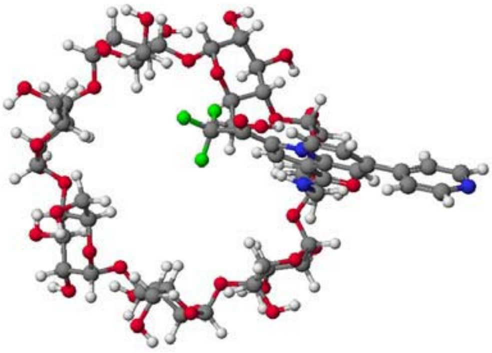
Table 4. Computed complexation energy, ΔE (kcal/mol), MM3

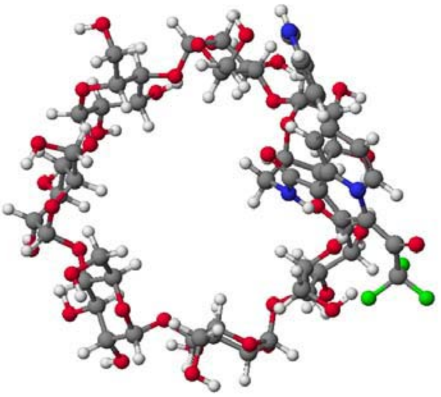
Complex	ΔE	Complex	ΔE	Complex	ΔE
S_1–B	–11.65	S_1–T_1	–11.68	S_1–T_2	–12.46
S_2–B	–10.12	S_2– T_1	–11.02	S_2– T_2	–11.28
S_3–B	–11.02	S_3– T_1	–12.26	S_3– T_2	–12.08
S_4–B	–11.29	S_4– T_1	–11.86	S_4– T_2	–12.82
S_5–B	–10.98	S_5– T_1	–9.53	S_5– T_2	–9.56
S_6–B	–12.46	S_6– T_1	–14.05	S_6– T_2	–14.34

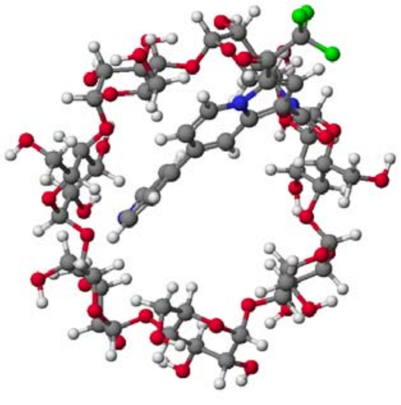
Within this scope, we then evaluated the energy gain upon association of guest molecule (benzene and toluene) with sensor conformers $S_{1–6}$ supposing a 1:1 host–guest complex. Each guest was moved along the C7 symmetry axe of the genuine cavity. Translation, rotation and inclination of the guest in the cavity were freely allowed. No regioselectivity has to be explored for benzene, but inclusion of toluene should be investigated in two directions: one with the methyl group remaining at the secondary rim of the cyclodextrin (T_1 conformations) and another for which the methyl group is pointed towards the cavity (T_2 conformations).


S1
ΔH = –1706.3339 kcal/mole


S2
ΔH = –1712.0146kcal/mole


S3
ΔH = –1714.1434kcal/mole


S4
ΔH = –1715.3390kcal/mole


S5
ΔH = –1709.2178 kcal/mole

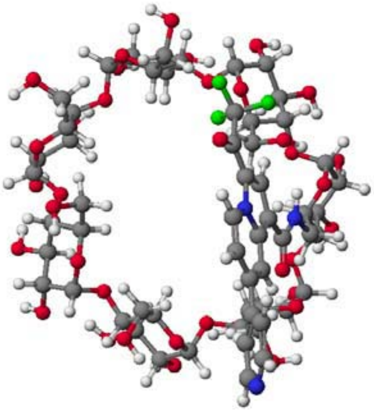
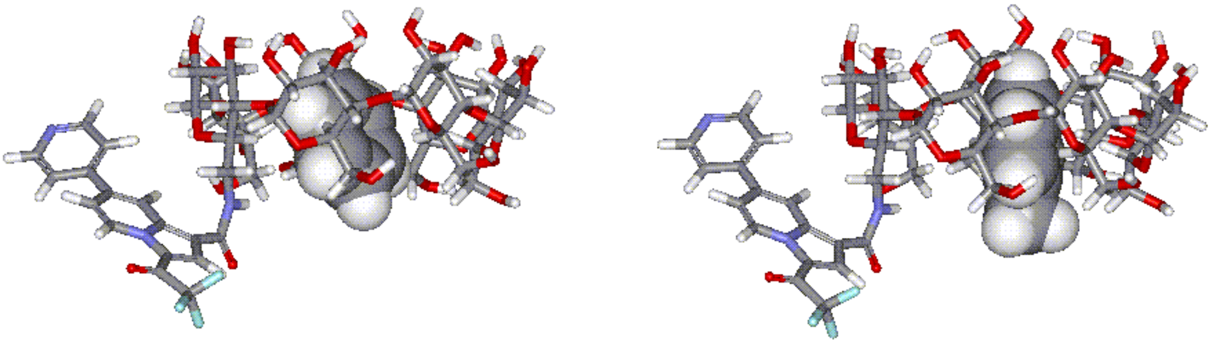

S6
ΔH = –1716.8993 kcal/mole

Figure 3. Structure and heat of formation (kcal/mol) of sensor **3** predicted by AM1 in water.

For each complex, the most stable structure was examined in term of computed stabilization energy ΔE, which was defined as the difference between the inclusion compound energy and the sum of the two individual energies for both sensor and guest (Table 4).

According to the enthalpy/entropy compensation phenomena, it has to be mentioned that stabilization energies are overestimated if compared to experimental stabilization. Besides, one can reasonably think that the differences between the S_1 to S_6 complexes are too low to compensate the most stable energy of the S_1 conformers when kept alone in water (favored with at least 3 kcal/mol). It should be noted that only the S_6 conformer allows a better recognition of benzene and toluene, but this sensor conformation is the less probable in water. As a consequence, S_1–B and S_1–T2 should be the most representative picture of benzene and toluene inclusion compounds (Figure 4).



S1–B conformation S1–T2 conformation

Figure 4. Structure of the stable conformations S1–B and S1–T2 for the complex of benzene and toluene respectively (guest in CPK model).

It should also be emphasized that the S_5 conformation leads to the poorer recognition. This may be attributed to the fact that S_5 corresponds to the most capped structure, and thus that the fluorescent moiety disturbs the complexation if this one is too close to the cyclodextrin cavity. If this assumption is right, it could also explain the good inclusion ability of the S_1 conformer: indeed, S_1 constitutes the most open structure, and thus the less disturbing one. At last, the weak sensing ability of sensor **3** may be due to the important distance which exists between the guest and the indolizine part of the sensor for the S_1 conformation: if few interactions occur between the two moieties, then the VOC's will have a small influence on the fluorescence intensity.

In the case of toluene, the fact that a better recognition is observed for the methyl group remaining at the primary rim of β–cyclodextrin may be justified by a more tight fit with this region. Indeed, the primary rim is narrower than the secondary rim. Nevertheless, differences between T1 and T2 stabilization energies are very weak, and both regioselectivity should contribute to the complexation.

The MM3 docking always predicts toluene to be more recognized than benzene, whatever sensor conformation is envisaged. This is consistent with our experimental results that showed a higher formation constant for toluene. The more tight fit of the methyl group with the narrower primary rim should be responsible of this behavior.

4 CONCLUSIONS

The fluorine fluorescent β–CD sensor **3** may be considered as a molecular sensor for VOCs. This host shows a pure monomer fluorescence, whose variation, although not very high, can be used as a parameter to describe the sensing ability. The theoretical study showed that the most probable sensor conformer presents an open cavity in water. Such open cavity seems to lead to a better recognition than the capped conformations. The experimental values of the constants are in agreement with computed complexation energies ΔE calculated by MM3 method, since both methods emphasized a greater stabilization of the toluene complex if compared to benzene.

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