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 from 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 and the formation constant values measured using a spectral displacement method (associated to a dedicated algorithmic treatment) are reported and confronted with the computed complexation energies. Only the open structures of the sensor seem to be involved in the aqueous complexation.

Key words: molecular docking, indolizine, β-cyclodextrin , VOCs, fluorescence detection, formation constant.

1 INTRODUCTION

Cyclodextrins are cyclic oligomers of D-glucopyranose, which can accommodate in their cavities a large variety of volatile organic compounds (VOCs). The modification of cyclodextrins with chromophore fragments has aroused interest because these derivatives can be expected to improve or alter their host-guest properties [1]. The fluorescent active cyclodextrins exhibit fluorescent sensor abilities for organic guest [2]. Recently, we reported the fluorescent host-guest systems using β-cyclodextrin modified with a indolizine fluorescent moiety [3]. Besides, he negligible cytotoxic effects of cyclodextrins are an important attribute in applications such as drug carrier, food and flavours, cosmetics, packing, textiles, separation processes, environment protection, fermentation and catalysis [4]. The synthetic procedure of the fluorine

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fluorescent indolizinic β-cyclodestrin (**3**) together with its sensing behaviour towards adamantanol (scheme 1) has been described in a previous paper [5].
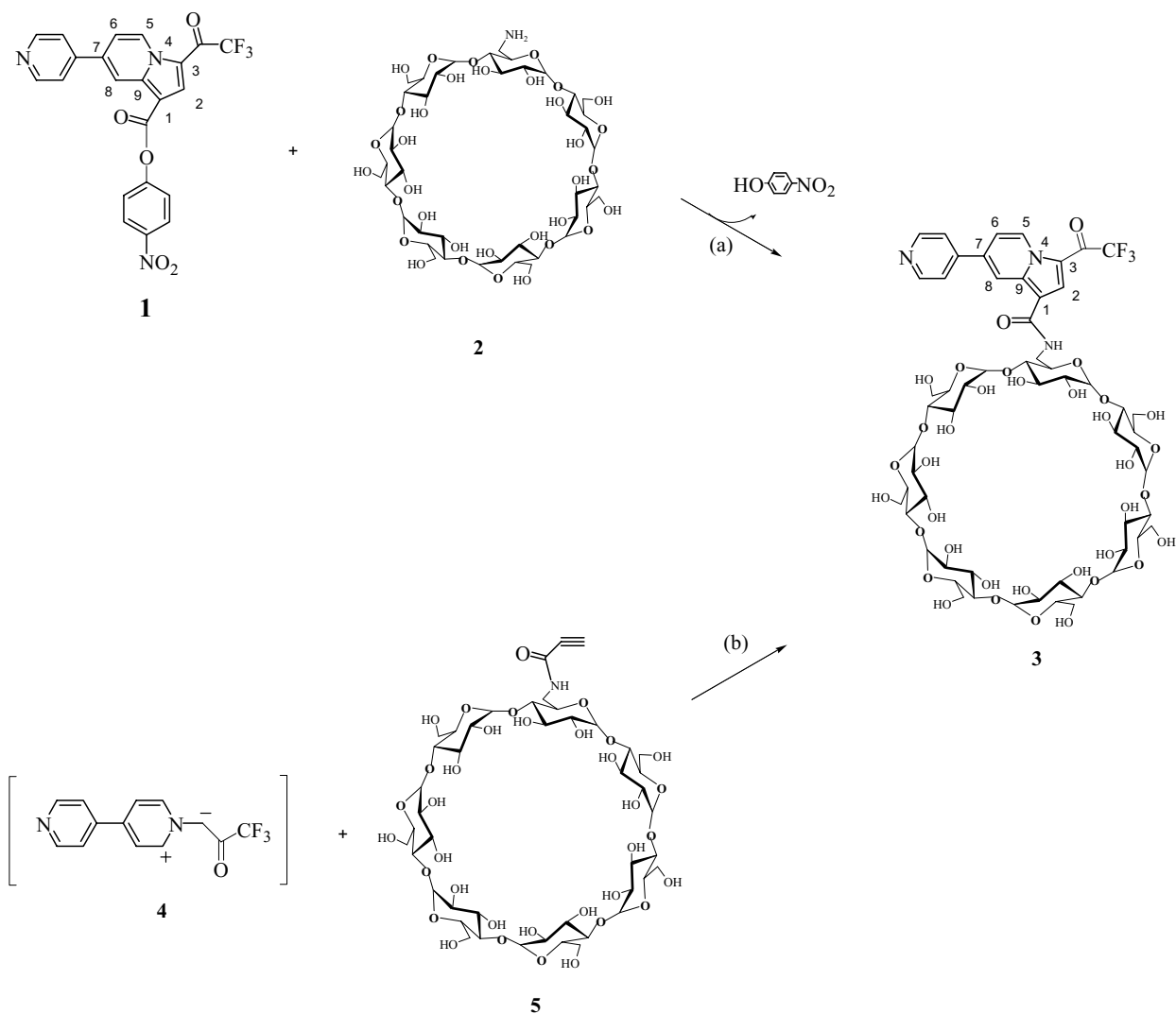
Scheme 1: Synthesis of the fluorescent indolizine β-cyclodextrin sensors

Thus, 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 VOCs and to see if the indolizine moiety can act as a fluorescent probe and as a hydrophobic cap to elevate the guest binding ability of the β-

cyclodextrin. In our study we choosed benzene and toluene as guest. As methods of investigation, we used a spectral displacement method for the determination of the formation constant by UV-visible spectroscopy, fluorescence to determine the sensitivity factor and molecular modelling by MM3 and AM1 methods for the calculation of computed formation energies.

2 MATERIALS AND METHODS

2.1 Visible Spectra

Spectra were recorded using a Perkin Elmer Lambda 2S double beam spectrometer and a quartz cell with optical path length of 1.00cm at 293K. All compounds were dissolved in phosphate buffer at pH 5.8.

For the spectral displacement method, the sensor was dissolved in methyl orange (MO) solution and each compound, benzene and toluene was dissolved in the resulting solution in order to avoid spectral variation due to a variation of the concentration of the solution.

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 characterised by a direct titration method. For a 1:1 molar ratio complex the calculation of formation constant K_f was developed as follows:
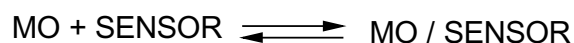
$$\text{MO} + \text{SENSOR} \rightleftharpoons \text{MO} / \text{SENSOR}$$

$$K_f = [\text{MO} / \text{SENSOR}] / [\text{MO}] [\text{SENSOR}] \tag{1}$$

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

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

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 applied to 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 determinated by applying a spectral displacement method with MO in its basic form 1:1:1: equilibrium MO: SENSOR: Guest (G).

MO + SENSOR + G ⇌ MO / SENSOR + G ⇌ 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 SNESOR cavity. The formation constant SENSOR / G 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 equilibrium 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 ands 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 spectre was 370 nm and excitation and emission slits were 4 nm.

2.4 Molecular Modelling

The sensor and guest molecules, as well as their complexes were 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 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 dihedral angle by rotational increments of 15°. The minimal values of ΔE are chosen according to the curve scribing. The analysis was developed tacking in account all single bounds composing the sensor **3**.
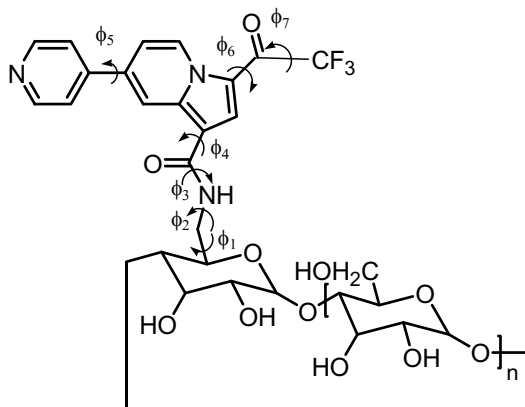
Scheme 2: Global structure of the sensor

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 concluded 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 minimised. Once the minima have been obtained by this MM3 search, each conformation is freely minimised according to AM1 hamiltonian in aqueous medium and gas phase. 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. Indeed, the non covalent interactions are easily reproduced than with semi empirical methods. The docking has been realised 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 occurred with the methyl group or the phenyl group first, we envisaged this two regioselectivity. For each complex, the theoretical parameter which has been calculated to evaluate the inclusion capacity of sensor **3** is the computed stabilisation 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 %.

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

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

In the same table are given, for comparison the similar formation constants involving the β-CD using the same experimental procedures described in this paper. These 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 (Fig.1) and by using an algorithmic procedure [6, 7]. The obtained values show that toluene is better recognized by the two cyclodextrins, but not in a significative way. 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.
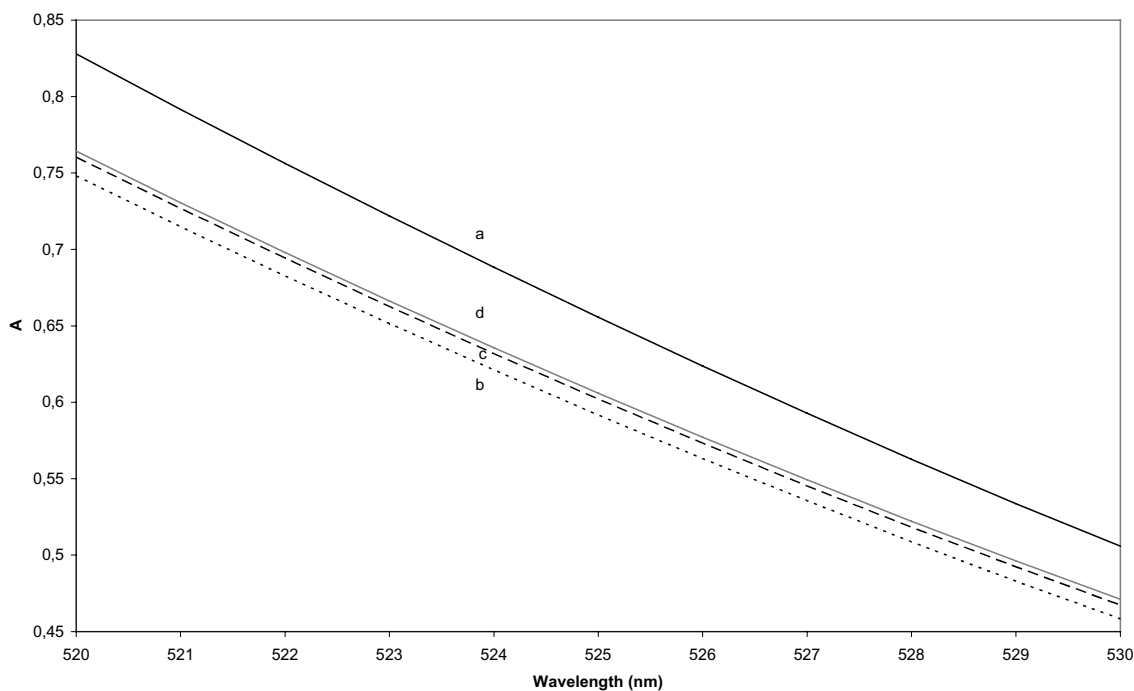
Figure. 1 : Absorption spectra for solutions containing (a) methyl orange 0.1mM, (b) methyl orange 0.1mM and sensor 0.1mM, (c) methyl orange 0.1mM and sensor 0.1mM and benzene 4.10mM, (d) methyl orange 0.1mM and sensor 0.1mM and toluene 4.13mM

The addition of benzene and toluene to a solution of sensor leads both to an increase of the fluorescence emission (Fig. 2). The fluorescence intensity of the modified cyclodextrin is affected by the presence of the guest molecule but as we can see on figure 2, saturation appears for a concentration of toluene about 6mM. So after such concentration the response of the sensor will not be concentration dependant. Moreover, for the two guest, the variation of intensity is very low ($\Delta I = 20$ for toluene) compared to other sensor ($\Delta I = 300$ for toluene) even if the formation constant of these two sensor with the guest are in the same order of magnitude [10].
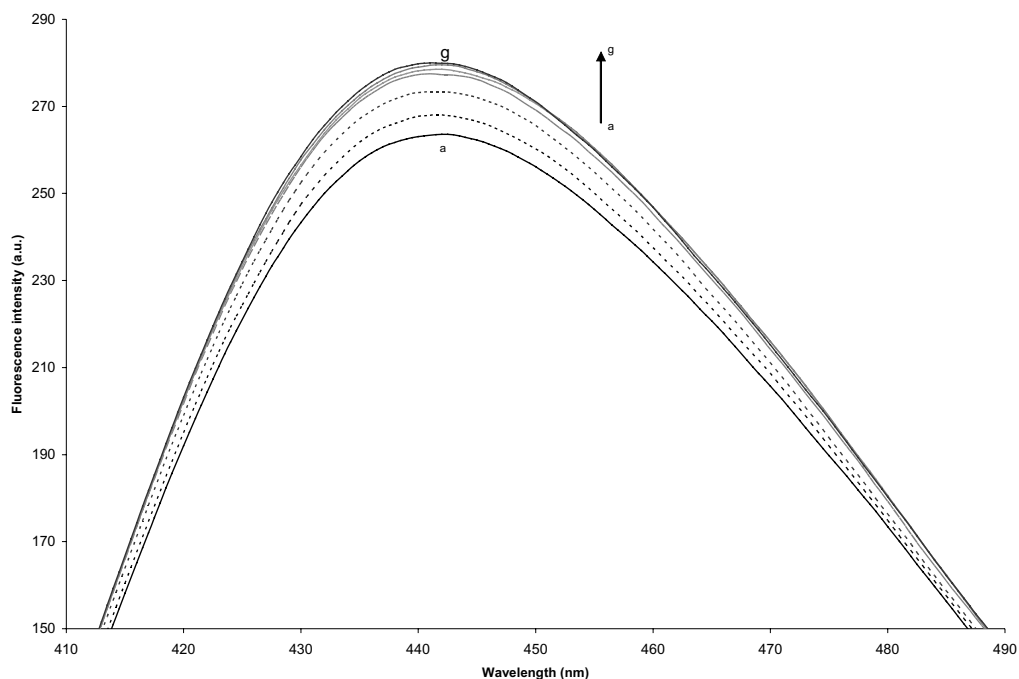
Figure 2 : Fluorescence spectra of the sensor in aqueous solution (a) (0.1mM, 25°C), at various concentrations of toluene (b) 1.9mM, (c) 3.8mM, (d) 5.7mM, (e) 7.6mM, (f) 9.5mM, (g) 19mM

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 nm, and I is a mixture of a host and a guest [1, b].

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

Table 2 : Sensitivity factor for benzene and toluene
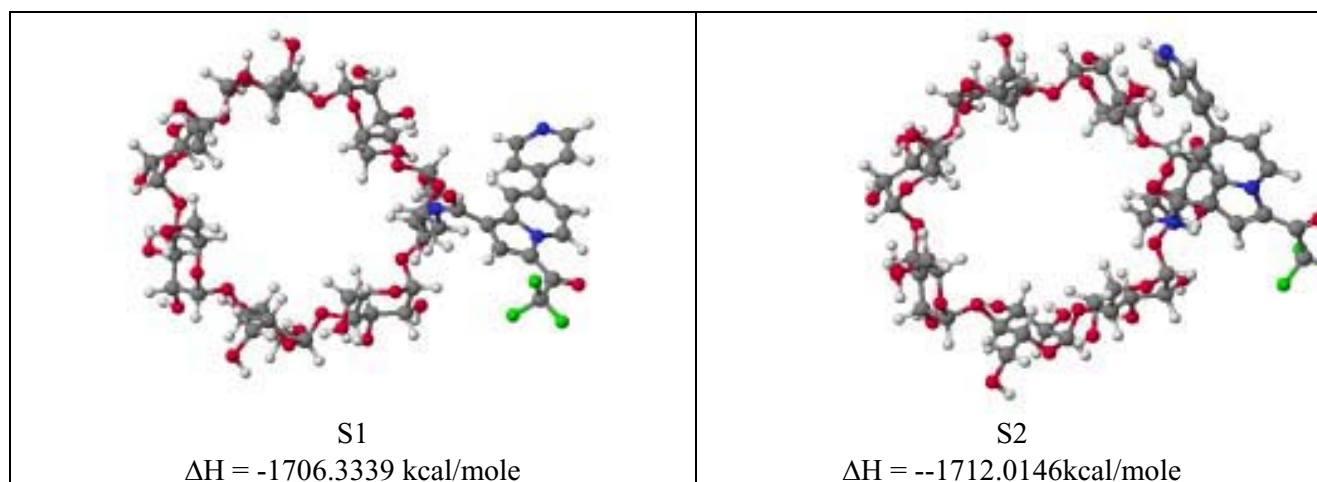
3.2 Theoretical Study

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 which control the relative position between the

fluorescent and cavity moieties. Each structure obtained by the MM3 search has then been minimised 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.

	ΔH_s kcal/mol (H_2O), AM1	ΔH kcal/mol (gaz), 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 3 : Enthalpy Formation

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 recommends the conformer S_6 as most stable, the water solvent calculation indicates the conformer S_1 as the most probable (Table 3). This result was predictable since S_1 structure presents the wider solvent surface accessible area, in opposition to capped structures which 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 affords the greatest contribution to the affinity of the studied host-guest inclusion compounds.

S1	S2
ΔH = -1706.3339 kcal/mole	ΔH = --1712.0146kcal/mole

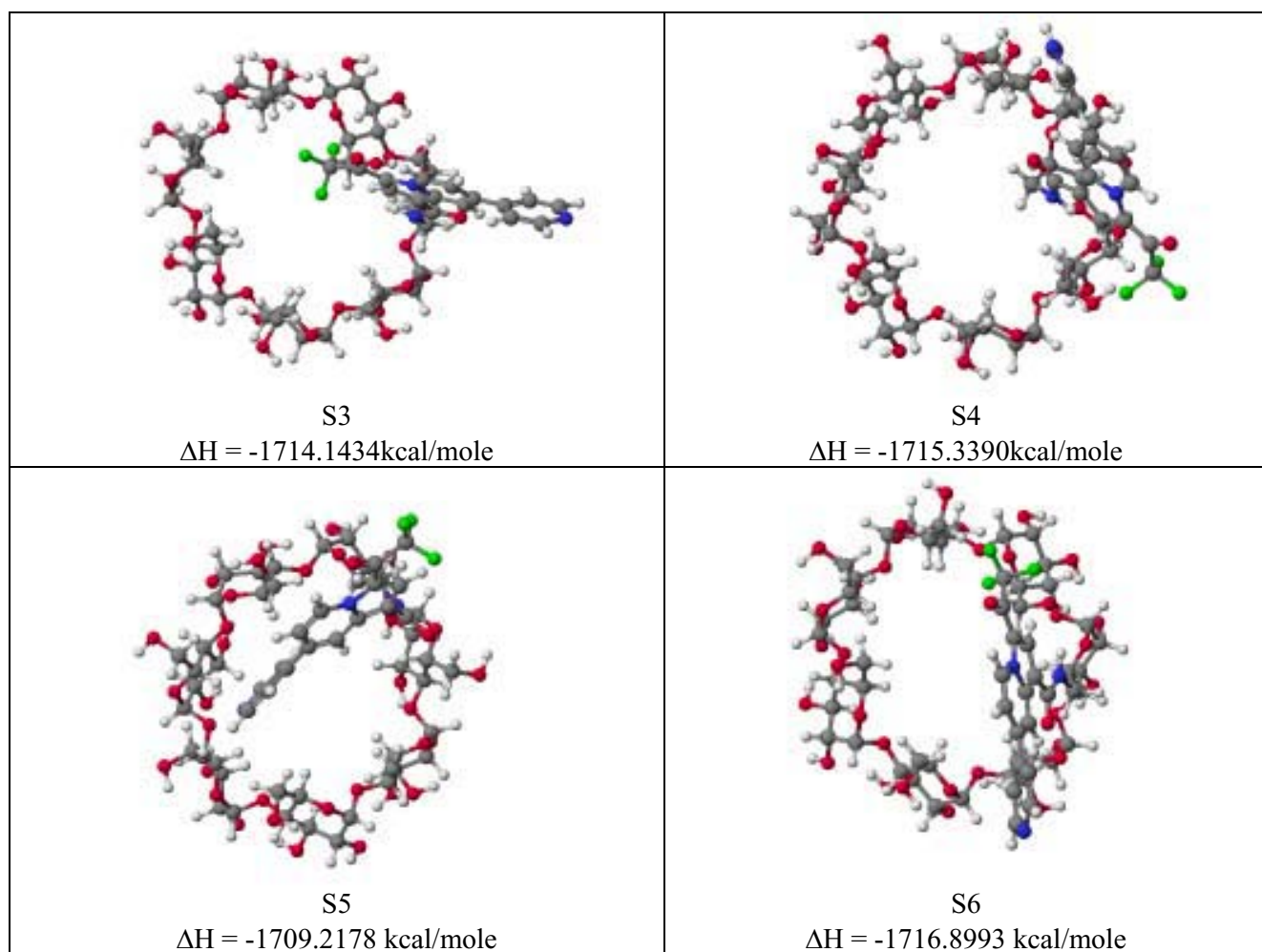|	
S3	S4
$\Delta H = -1714.1434$ kcal/mole	$\Delta H = -1715.3390$ kcal/mole
S5	S6
$\Delta H = -1709.2178$ kcal/mole	$\Delta H = -1716.8993$ kcal/mole

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

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). 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).

complexe	ΔE	complexe	ΔE	complexe	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

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

According to the enthalpy/entropy compensation phenomena, it has to be mentioned that stabilisation energies are overestimated if compared to experimental stabilisation. Thus, 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 (favoured with at least 3 kcal/mol). Besides, 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. It should also be emphasised 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.

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 face. Indeed, the primary rim is narrower than the secondary rim. Nevertheless, differences between T1 and T2 stabilisation energies are very weak, and both regioselectivity should contribute to the complexation.

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

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 in water the most probable sensor conformer presents an open cavity. 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 emphasised a greater stabilisation of the toluene complex if compared to benzene.

5 REFERENCES

[1]   (a) J. Szejtli, Cyclodextrin technology, Kluwer, Dordrecht (1988) (b) M. Narita, S. Koshizaka, F. Hamada, *J. Inclusion Phenom.,* 35, 605-619 (1999).

[2]   (a) F. Hamada, K. Ishikawa, J. Tamura, A. Ueno, *Anal. Sci.*, 11, 935 (1995) (b) F. Hamada, K. Ishikawa, Y. Higuchi, Y. Akagami, T. Osa, *J. Inclusion Phenom. Mol. Recog. Chem.*, 25, 283 (1996) (c) F. Delattre, P. Woisel, F. Cazier, P. Decock, G. Surpateanu, *Int. Elec. J. Mol. Design*, 3, 9 (2004).

[3]   (a)F. Delattre, P. Woisel, G. Surpateanu, M. Bria, F. Cazier, P. Decock, *Tetrahedron*, 60 (7), 1557-1562 (2004) (b) M. Becuwe, F. Delattre, G.G. Surpateanu, F. Cazier, P. Woisel, G. Garçon, P. Shirali, G. Surpateanu, *Heterocycl. Commun*., HC 040602 (in print) (c) F. Delattre, P. Woisel, G. Surpateanu, F. Cazier, P. Blach, *Tetrahedron*, ref.6649 (2004) (submitted).

[4]   E.M. Martin Del Valle, *Process Biochemistry*, 39, 1033-1046 (2004).

[5]   N.C. Lungu, A. Depret, F. Delattre, G.G. Surpateanu, F. Cazier, P. Woisel, P. Shirali, G. Surpateanu, *J. Fluorine Chem.*, 20040617/1 (2004) (submitted)

[6]   D. Landy, S. Fourmentin, M. Salome, G. Surpateanu, *J. Inclusion Phenom. Macrocycl. Chem.*, 187-196 (2000).

[7]   D. Landy, S. Fourmentin-Lamotte, F. Elhoujajji, G. Surpateanu, *Proceeding of the 9th International Symposium on Cyclodextrins*, Kluwer Academic Publishers, J.J. Torres Labandeira and J.L. Vila-Jato ed., 663-666 (1998).

[8]   CAChe, Work System, 6.01 (2003).

[9]   (a) G.G. Surpateanu, G. Vergoten, G. Surpateanu, *J. Mol. Struct.*, 526, 143-151 (2000) (b) G.G. Surpateanu, G. Vergoten, G. Surpateanu, *J. Mol. Struct.*, 559, 263-271 (2001) (c) G.G. Surpateanu, F. Delattre, P. Woisel, G. Vergoten, G. Surpateanu, *J. Mol. Struct.*, 645, 29-36 (2003).

[10]   F. Delattre, *PhD dissertation,* Dunkerque, (2003).