Experimental and Theoretical Study on the Inclusion Capability of a Fluorescent Indolizine β-Cyclodextrin Sensor Towards Volatile and Semi-volatile Organic Guest

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Abstract

Inclusion equilibria of a new fluorescent indolizine modified β-cyclodextrin were studied in aqueous solution to evaluate its use as a fluorescent chemo-sensor for volatile organic compounds (VOCs). The host compound shows a decrease of the fluorescence intensity by adding adamantanol, benzene, toluene, phenol and *p*-cresol as guest. The sensing parameter ($\Delta I/I_0$) was used to show the sensing ability of the host. The formation constant values measured using a spectral displacement method and a specific algorithm treatment are reported. Although the guest binding ability of the sensor is not enhanced by the existence of the hydrophobic cap, this new cyclodextrin sensor shows a very strong sensing ability. The experimental values of the constants were in good agreement with the computed complexation energies, ΔE, from molecular mechanics modelling of the inclusion process.

Introduction

During the past decade, much effort has been devoted to develop analysis systems with which chemical and biological compounds can be detected without any chemical modification of analytes [1–5]. Absorption and fluorescence changes of appropriately designed hosts associated with guest binding are important in this respect.

Volatile organic compounds (VOCs) are widely used in industry and can present serious medical, environmental, and explosion dangers. VOC sensors are therefore commonly used to monitor oil-drilling platforms, fuel storage tanks, air pollution sources, and other locations where VOC leaks may occur. To this end we are developing sensors for the detection of VOC in personal exposure or pollution control.

Supramolecular chemistry can be utilized for chemical sensor technology with compounds like cyclodextrins (CD), paracyclophanes and calixarenes wich are able to form host–guest complexes. In particular, cyclodextrins are used for gas phase measurements [6]. Cyclodextrins are cyclic oligosaccharides composed of at least six α-D-glucose subunits forming a truncated cone. The primary hydroxyl groups are directed to the narrow side and the secondary hydroxyl groups are on the wide side of the torus. This results in

a hydrophobic interior and a hydrophilic outer surface. The hydrophobic cavity of the cyclodextrins allows the formation of inclusion complexes with a variety of organic compounds in aqueous solution [7–9]. Binding specificity depends predominately on the guest size and geometry, it is dependant upon noncovalent forces such as Van der Waals forces, hydrogen bondings and hydrophobic interactions. Such compounds may be useful for constructing a molecule-sensing system. The inclusion ability of cyclodextrin derivatives towards aromatic volatile organic compounds is well known [10, 11].
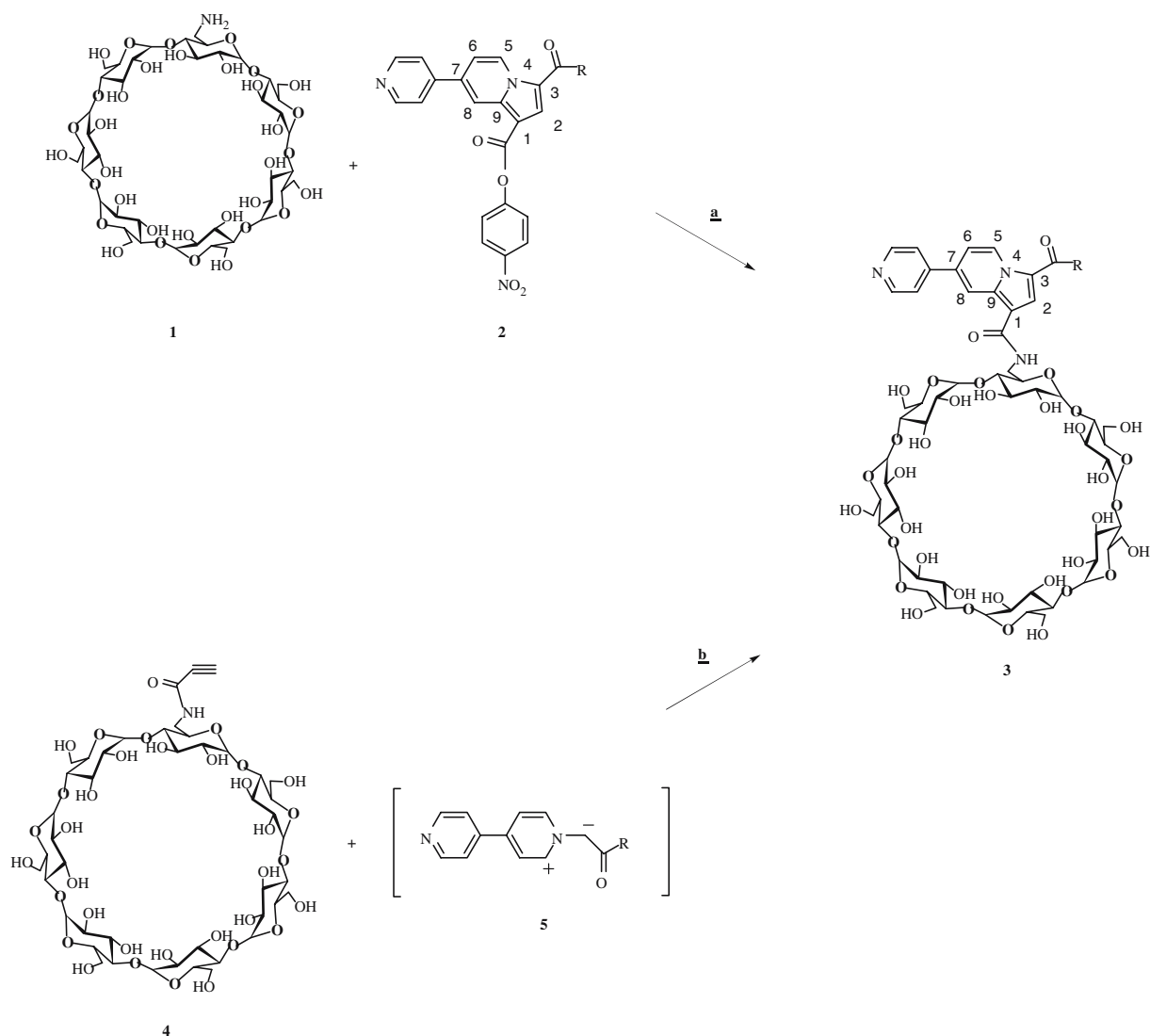
The synthesis of a new class of fluorescent sensors based on β-cyclodextrin fragment (β-CD) and indolizine unit was reported in previous studies [12, 13].

Two different synthetic ways were employed (Scheme 1, 2). The first (a) involves an amidation of 6-deoxy-6-amino-β-cyclodextrin **1** with an esteric indolizine derivative **2**. The second (b) results in the same sensors **3** obtained by a 3 + 2 cycloaddition reaction between ylide 5, generated ''*in situ*'' from its corresponding salt, and 6-propynamido β-cyclodextrin **4**.

By both synthetic procedures a and b, we synthesized the fluorescent fluorine sensor **3** ($R = 4\text{-}C_6H_5\text{-}F$) [14].

The attachment of fluorophores to synthetic or natural receptors has already received considerable interest in endeavours to furnish new molecular sensor [15–20]. In particular, fluorescent cyclodextrins have generated

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Scheme 1. Synthesis of fluorescent indolizine β-cyclodextrin sensors.
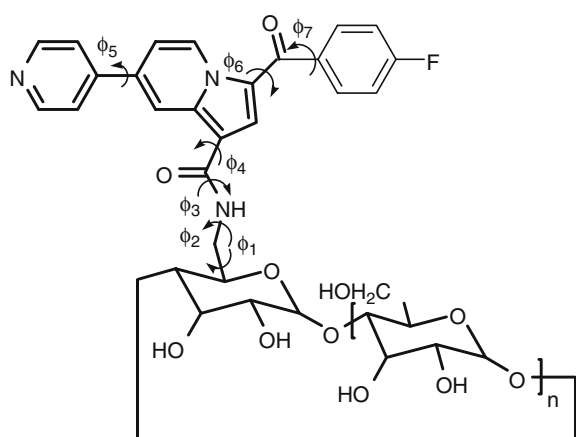
Scheme 2. Global structure of sensor 3.

interest as biologically active products and they present fluorescent properties [29–31].

The chemo-sensor ability of our new host molecule towards VOCs was studied by fluorescence. A spectral displacement method for formation constant determination by UV–Vis spectra was performed to examine if the indolizine moiety could act as a hydrophobic cap to elevate the guest binding ability of the β-cyclodextrin. Computed formation energies were calculated by MM3 and AM1 methods and compared with the obtained formation constant.

Materials and methods

Chemicals

Benzene, toluene, phenol, *p*-cresol, adamantanol, methyl orange, sodium hydroxide and potassium dihydro-genophosphate (Aldrich) all of analytical reagent grade

attention from the synthetic community as witnessed by recent articles dealing with their synthesis and emphasizing their sensory [21–27] but also their biochemical properties [4, 28]. The indolizinic derivatives are of

were used as received. Deionised water was used throughout this work.

Visible spectra

Spectra were recorded using a Perkin Elmer Lambda 2S double beam spectrometer and a quartz cell with optical path length of 1.00 cm at 293 K. All compounds were dissolved in phosphate buffer at pH 5.8. The control of temperature was realised by the use of a thermostated bath linked to the cell holder (accuracy: ±0.1 °C). The stability of the complexes formed between methyl orange and the macrocyclic hosts (sensor and β-cyclodextrin) is first obtained by the use of the direct titration method. The complexing ability of both hosts were evaluated for benzene, toluene, phenol and *p*-cresol by means of a spectral displacement method with methyl orange. Dedicated algorithmic treatments were applied to the first derivatives of UV spectra in order to avoid any spectral influence of diffraction phenomena [32–33]. Spectra were recorded between 520–530 nm for a MO concentration fixed at 0.1 mM. This wavelength range corresponds to the optimal spectral variation between the free and complexed forms of MO.

Fluorescence measurements

The measurements were carried out with a Perkin Elmer LS-50B fluorimeter at 293K. The excitation wavelength of the fluorescence spectra was 370 nm and excitation and emission slits were 4 nm. The spectra were collected from 300 nm to 700 nm with a fixed scan rate of 100 nm/min. The control of temperature is realised by the use of a thermostated bath linked to the cell holder (accuracy: ±0.1 °C).

Molecular modelling

The sensor and guest molecules were built starting from the data provided by the Structural Data Base System of the Cambridge Crystallographic Data Center. The various structural manipulations were made using the CAChe Library [34] on a PC-Computer.

Sensor conformation
A general procedure of multiconformational search has been used with the MM3 force field. The energetic variation is recorded in function of the dihedral angles controlling the relative position between the cyclodextrin cavity and the florescent moiety (rotational increments of 15° for each variable). The analysis was developed taking in account all single bounds composing the sensor **3**. Nevertheless, the proximity of the toroïdal cycle of β-CD restricts the variation of the ϕ_1 and ϕ_2 dihedral angles. In addition, the torsions according to ϕ_5, ϕ_6 and ϕ_7 do not control directly the position of the fluorescent fragment in respect to the primary face of β-CD. Hence, no systematic variation is applied to the ϕ_1, ϕ_2, ϕ_5, ϕ_6 and ϕ_7 dihedrals, which are

only energy minimised. The rotations assigned to ϕ_3 et ϕ_4 dihedrals are thus considered as the key variables controlling the sensor structure, and only these two dihedrals undergo systematic variations.

Inclusion compounds conformation
The docking of each guest into the β-CD unit has been performed using a dummy atom, centrally placed in the cyclodextrin cavity (in the mean plane of glucosidic oxygens). Each orientation has been taken in consideration for each guest. Three parameters were varied to explore the conformational space of the inclusion compound: the distance between host and guest, the orientation of the aromatic ring inside the host cavity, and its tilt angle. For this purpose, a multiconformational search has been employed with the MM3 force field. The most stables structures obtained by this procedure are then energy minimised without any constraint. The difference ΔE, kcal/mol) between the total energy of the inclusion complex and the sum of their individual components in their optimized ground states was then used as the theoretical parameter to evaluate the inclusion ability of sensor **3**.

Results and discussion

Experimental study

In order to evaluate the inclusion ability of our new sensor, a UV-visible competition method was used to study the inclusion of benzene, toluene, phenol and *p*-cresol. Such a method is based on the spectral variation observed upon addition of each guest on a solution containing both the sensor molecule and methyl orange. It implies that the SENSOR/MO complex should be characterised. A titration has thus been realised for this system, and as can be seen from the fit appearing in Figure 1, the obtained data are in agreement with a 1:1
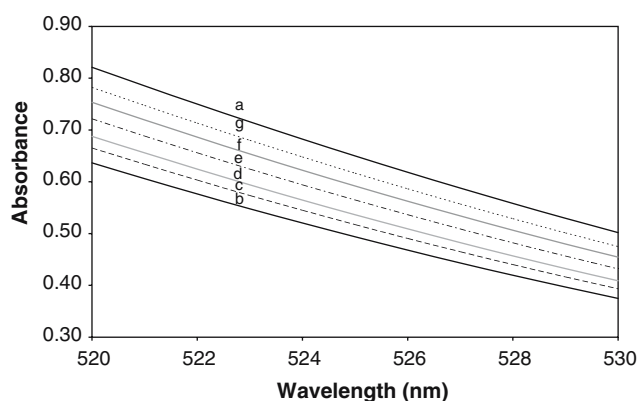

Figure 1. Absorption spectra for solutions containing (a) methyl orange 0.1 mM, (b) methyl orange 0.1 mM and Sensor 0.1 mM (c) methyl orange 0.1 mM and Sensor 0.083 mM, (d) methyl orange 0.1 mM and Sensor 0.067 mM (e) methyl orange 0.1 mM and Sensor 0.05 mM (f) methyl orange 0.1 mM and Sensor 0.033 mM and (g) methyl orange 0.1 M and Sensor 0.017 mM.
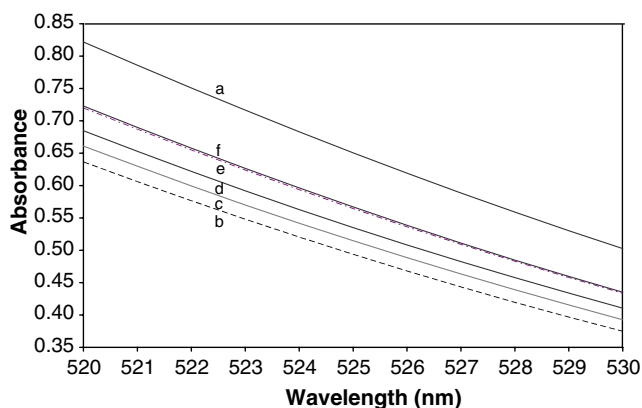
Figure 2. Absorption spectra for solutions containing (a) methyl orange 0.1 mM, (b) methyl orange 0.1 mM and Sensor 0.1 mM, (c) methyl orange 0.1 mM, Sensor 0.1 mM and benzene 4.35 mM, (d) methyl orange 0.1 mM, Sensor 0.1 mM and toluene 6.95 mM, (e) methyl orange 0.1 mM, Sensor 0.1 mM and phenol 13 mM and (f) methyl orange 0.1 mM, Sensor 0.1 mM and *p*-cresol 4.8 mM.

Table 1. Formation constants (M^{-1}) determined in this study by UV–vis spectroscopy compared to literature data

Guest	Present study		Literature[35–38]
	SENSOR	β-CD	β-CD
MO	1750 ± 170	2500 ± 240	2050[35]
Benzene	53 ± 6	82 ± 9	120[36] – 194[37]
Toluene	55 ± 6	102 ± 10	140[36] – 214[37]
Phenol	85 ± 9	115 ± 11	95[38]
p-Cresol	215 ± 20	195 ± 20	249[38]

complex ratio. The experiment allowed an association constant of 1750 M^{-1} to be determinated for the SENSOR/MO inclusion compound. This value is slightly lower than for the corresponding genuine cyclodextrin complex (2500 M^{-1}).

When applying the competition method to the sensor, one can observe an increase of absorption (Figure 2), which indicates the formation of inclusion compounds.

The obtained spectral variations are also in agreement with the 1:1 host/guest ratio, as could be expected from the analogous results for original β-CD. The formation constants are calculated by an algorithmic procedure [32, 33] and are reported in Table 1. The stabilities involving the genuine β-CD (estimated by the same experimental procedure) are also presented and compared to values found in literature using other experimental procedures [35–38]. One can notice from Table 1 that the formation constants of organic guests with our sensor are in the same order of magnitude than those involving β-cyclodextrin. Hence, the guest binding ability of the sensor is not enhanced by the existence of the hydrophobic cap, but this may not be essential to present a good sensing ability.

As a consequence, we have studied the fluorescence variation induced by addition of various concentrations
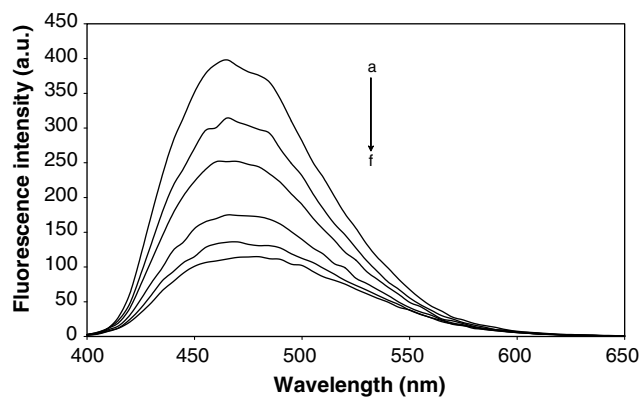

Figure 3. Fluorescence spectra of (a) the sensor in aqueous solution (0.1 mM, 25 °C), at various concentrations of toluene (b) 2.7 mM, (c) 5.4 mM, (d) 13.5 mM, (e) 18.9 mM, (f) 27 mM.

of each guest to an aqueous sensor solution. The corresponding curves are illustrated in the case of toluene in Figure 3.

The addition of toluene leads to a decrease of the fluorescence intensity, without any significant shift of the maximum emission wavelength. This trend is also observed for benzene, phenol and *p*-cresol. Since the fluorescent intensity of the modified cyclodextrin is strongly affected by the presence of these guest molecules, it may be used as fluorescent sensor for volatile or semi-volatile organic molecules. Within this scope, the $\Delta I/I_0$ value was used as the sensitivity factor to illustrate the sensing abilities of the sensor (ΔI is $I - I_0$, where I_0 is the fluorescence intensity of the host alone at 465 nm, and I corresponds to a mixture of host and guest [24]). The corresponding data are summarised in Table 2.

Such values are particularly interesting, since they are significantly higher than in a previous study [39]. The introduction of the fluorophenyl substituent thus enhances the detection ability of this series of fluorescent sensor. In addition, since the volatile guests of the present work have not been extensively studied in the cyclodextrin sensing field, we also investigated the influence of adamantanol on the fluorescence behaviour of such sensor. Indeed, adamantanol may serve as a reference compound since it is widely used in complexation studies because of its important affinity for β-cyclodextrins. As can be seen from Figure 4, the addition of adamantanol again leads to a decrease of the fluorescence intensity, but also to a bathochromic effect.

The $\Delta I/I_0$ obtained in presence of 0.25 mM adamantanol is equal to $-0,53$ at 370 nm. To the best of our

Table 2. Fluorescence sensitivity factors ($\Delta I/I_0$) for the sensor in presence of different guest at 293 K in aqueous solution

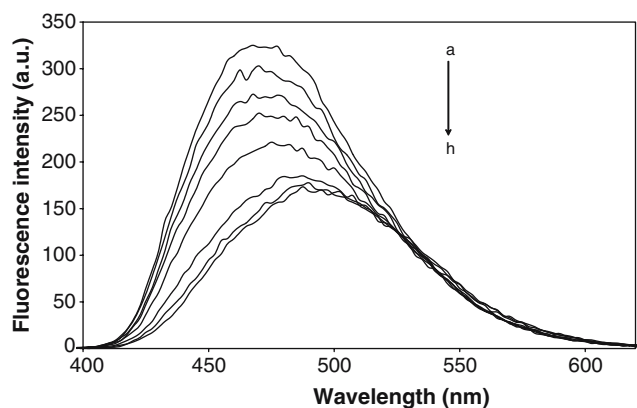| Guest	$\Delta I/I_0$
Benzene	-0.54
Toluene	-0.67
Phenol	-0.66
p-Cresol	-0.65

Figure 4. Fluorescence spectra of (a) the sensor in aqueous solution (0.08 mM, 25 °C), at various concentrations of adamantanol (b) 0.002 mM, (c) 0.008 mM, (d) 0.0125 mM, (e) 0.02 mM, (f) 0.05 mM, (g) 0.08 mM and (h) 0.2 mM.

knowledge, this may be the strongest $\Delta I/I_0$ for such compound, thus underlining the very good sensing ability of this new cyclodextrin sensor.

Theoretical study

A multiconformational search has been realised on the sensor alone and has been followed by final geometry optimizations with two stages: a preliminary minimization with the MM3 method, and then a AM1 minimization without imposing any restrictions. Among the two general types of sensor structure which could be identified, the open cavity type is more stable than its corresponding conformer with a capped cavity. The most stable structure (S_1) orients the fluorescent moiety in such a way that few interactions occur with the cyclodextrin cavity and that no self inclusion is observed (Figure 5).

It should be emphasised that the various dihedrals linking the cyclodextrin to the fluorescent adduct are only constrained by the cyclodextrin steric influence, so that the dynamical behaviour of such sensor should lead to the coexistence of many open conformations. The S_1 conformation thus only represents an instantaneous picture of our modified β-CD, but may show in what extent sensor **3** is able to make inclusion compound with organic guests. Within this scope, we evaluated the energy gain upon association of the guest molecules

(benzene, toluene, phenol and *p*-cresol) with sensor conformer S_1, taking into account the 1:1 host–guest complex. In other words, we calculated the stabilization energy ΔE due to the inclusion of guest in the inner cavity of the sensor **3**. The corresponding values and conformations are presented in Table 3 and Figure 6 respectively.

As the fluorescent moiety is kept outside of the cavity, there is no steric hindrance during the docking for any guest, which explains that the formation constants observed for sensor **3** were closed to those of the genuine β-CD, the fluorescent adduct having little influence on the complexation. Moreover, since Van der Waals interactions are known to be the dominant part of the energetic stabilisation, it was thus predictable that the greater the guest, the more stable the complex. Indeed, the inclusion of toluene is predicted to be more stabilised than benzene, while cresol is better recognised than phenol. Both hydroxylated compounds also present more negative ΔE than their corresponding apolar species. Such results are in agreement with the experimental values, as the same order of stability is obtained. Since the four guests present similar structure, the entropic component of the complexation is likely to be proportional to the enthalpic part, in accordance with the enthalpy/entropy compensation. Thus, it is not surprising that the calculated enthalpic variation ΔE may be qualitatively correlated to the formation constants.

Conclusion

A new indolizine modified β-cyclodextrin has been synthesized in order to investigate its fluorescent molecular sensing ability for VOCs. We have shown that the appended indolizine moiety of the host does not act as a hydrophobic cap, as there is no enhancement of the

Table 3. Computed energy of complexation, ΔE (kcal/mol)

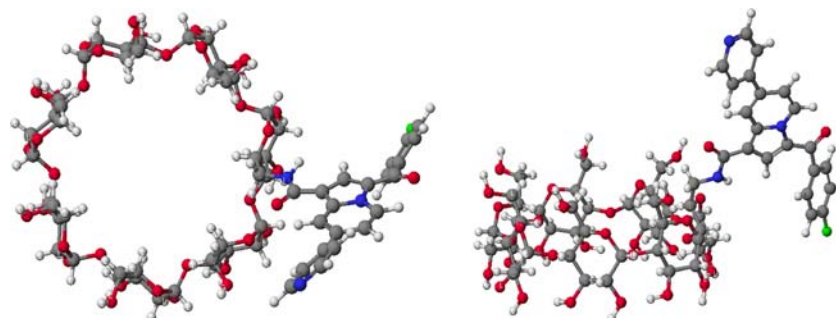| Guest	ΔE (kcal/mol)
Benzene	−7.2
Toluene	−8.9
Phenol	−11.4
p-Cresol	−12.7

Figure 5. Representation of the most stable conformer (S_1) of the sensor.
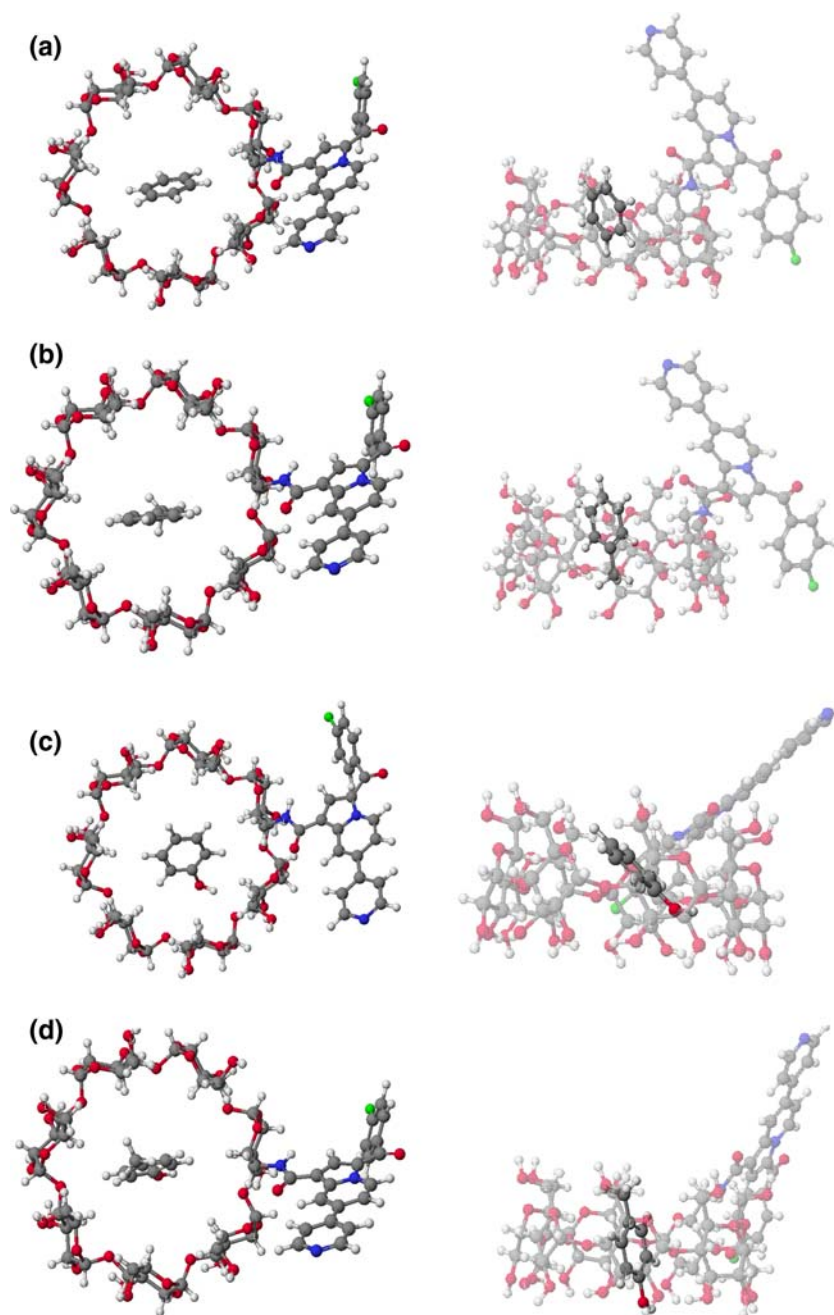
Figure 6. Representation of the top view and side view of the conformation of host–guest complexes (a) benzene, (b) toluene, (c) phenol and (d) *p*-cresol.

binding ability of the genuine *β*-cyclodextrin. This is in agreement with the molecular modelling of the sensor since open cavity conformers are the most stable and since few influence of the indolizine moiety has been recorded during the docking of the organic guests. Nevertheless, the fluorescence variation observed upon addition of volatile guests is particularly high and thus allows its use as a fluorescent sensor for organic molecules. The fluorophenyl substituent seems to greatly enhance the detection ability of this series of indolizine modified *β*-cyclodextrins. Since such fluorescent sensory system may be very useful to detect guests that are spectroscopically inert, the present study will be extended towards other volatile organic compounds.

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