

Supporting Information

**Host-Guest Interaction of Coumarin-Derivative Dyes
and Cucurbit[7]uril: Leading to the formation of
Supramolecular Ternary Complexes with Mercuric
Ions.**

Margarita E. Aliaga,^{a,*} Luis García-Río,^b Márcia Pessêgo,^b Rodrigo Montecinos,^a
Denis Fuentealba,^a Iván Uribe,^a Manuel Martín-Pastor^c and Olimpo García-Beltrán.^d

^a Facultad de Química, Pontificia Universidad Católica de Chile, Casilla 306,
Santiago 6094411, Chile.

^b Departamento de Química Física, Centro de Investigación en Química Biológica y
Materiales Moleculares (CIQUS), Universidad de Santiago, 15782 Santiago, Spain.

^c Unidade de Resonancia Magnética, RIAIDT, Edif. CACTUS, Universidad de Santiago,
15782 Santiago, Spain.

^d Facultad de Ciencias Naturales y Matemáticas, Universidad de Ibagué, Carrera 22
Calle 67, Ibagué 730001, Colombia.

* Corresponding author. Tel.: +56-(2)-23547126; Fax: +56-(2)-26864744. E-mail
address: mealiaga@uc.cl.

Contents	page
Synthetic procedures and characterizations of compounds 1 and 2 .	S-3
Figure S1. Normalized UV-vis absorption spectra of; (A) coumarin-derivative dye 1 (2 μM) in water (a), and (b) water in the presence of CB7 (30 μM), (B) coumarin-derivative 3 dye (2 μM) in water (a), and water in the presence of CB7 (b) 2 μM and (c) 30 μM .	S-5
Figure S2. Normalized excitation and emission spectra of coumarin derivative dyes: 1 (A) and 2 (B).	S-5
Figure S3. Time-resolved fluorescence decay profiles of the dye 1 (5 μM) at pH=5.0 (1) and at pH=2.0 (2); the 1 -CB7 complex at pH=5.0 (3) and at pH=2.0.	S-6
Figure S4. Time-resolved fluorescence decay profiles of the dye 2 (5 μM) at pH=5.0 (1) and at pH=2.0 (2); the 2 -CB7 complex at pH=5.0 (3) and at pH=2.0.	S-6
Figure S5. ^1H -NMR titration of TPA-CB7 complex with dye 2 .	S-7
Figure S6. Partial ^1H NMR spectra (750 MHz) for tetrapropylammonium bromide (TPA) 0.15 mM in DCl (10%)-D ₂ O.	S-7
Figure S7. ^1H NMR titration experiments of the dye 2 using competitive displacement of tetrapropylammonium bromide (TPA) from CB7.	S-8
Figure S8. Partial ^1H NMR spectra (750MHz) comparison for: (A) 1 alone in DCl (10%)-D ₂ O and (B) 1 and CB7 (3 eq).	S-8
Figure S9. Benesi-Hildebrand plots for dye 1 by fluorescence emission (A) and dye 2 using UV/Vis absorbance (B) with added Hg ²⁺ . The symbols represents: (○) solutions containing the dye in the absence of CB7 and (●) in the presence of CB7 (both at pH = 2).	S-9
Table S1. Apparent binding constants for mercuric ions binding to coumarin-derivative dyes alone and complexed by cucurbit[7]uril.	S-9
Determination of apparent binding constants for the 1:1 complex.	S-9
References	S-11

Synthetic procedures and characterizations of dyes 1 and 2.

Synthesis of 7-(Diethylamino)-N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-2-oxo-2H-chromene-3-carboxamide (1) and N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-11-oxo-2,3,5,6,7,11-hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxamide (2). Salicylaldehyde derivatives were condensed with ethyl malonate giving ethyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate.¹ This coumarin was condensed with 2-amino-2-(hydroxymethyl) propane-1,3-diol (TRIS) to afford (1 and 2), by analogy with a literature procedure.^{2,3}

7-(Diethylamino)-N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-2-oxo-2H-chromene-3-carboxamide (1). 4-Diethylaminosalicylaldehyde (9.00 g, 46.6 mmol), diethyl malonate (8.00 g, 50 mmol), piperidine (0.5 mL) and one drop of AcOH, were mixed in absolute ethanol (60 mL) and refluxed for 6 hours. The solution was cooled to room temperature and poured onto 200 mL of ice. The precipitate formed was filtered, washed with cold water:ethanol 1:1, and dried to give the desired product ethyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate. A solution of ethyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate (3.0 g, 10.37 mmol) and 2-amino-2-(hydroxymethyl)propane-1,3-diol (TRIS) (1.33 g, 11.0 mmol) in 30 mL of EtOH was stirred and refluxed for 20 h. The precipitate formed was filtered and washed with hot EtOH, yielding the compound (1) as light yellow crystals (2.83 g, 74.9 %); mp 159-161 °C; ¹H NMR (400 MHz, DMSO-*d*₆): 9.02 (s, 1H), 8.66 (s, 1H), 7.66 (d, *J*=8.0, 1H), 6.79 (dd, *J*=8.6, 1.0, 1H), 6.62 (d, *J*=1.0, 1H), 3.63 (s, 6H), 3.47 (q, *J*=8.0, 4H), 1.13 (t, *J*=8.0, 6H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 12.7, 44.7, 57.0, 63.0, 96.2, 108.0, 109.9, 110.5, 131.9, 148.0, 152.8, 157.7, 162.1, 162.9. *m/z* 364.7.

N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-11-oxo-2,3,5,6,7,11-hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxamide (2). 8-hydroxy-1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinoline-9-carbaldehyde (1) (1.00 g, 4.6 mmol), diethyl malonate (0.96 g, 6.0 mmol), piperidine (0.25 mL) and one drop of AcOH, were mixed in absolute ethanol (30 mL) and refluxed for 6 hours. The solution was cooled to room temperature and poured onto 100 mL of ice. The precipitate formed was filtered, washed with cold water:ethanol 1:1, and dried to give the desired product ethyl 11-oxo-2,3,5,6,7,11-hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxylate. A solution of ethyl 11-oxo-2,3,5,6,7,11-hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-

ij]quinoline-10-carboxylate (1.0 g, 3.2 mmol) and 2-amino-2-(hydroxymethyl)propane-1,3-diol (TRIS) (0.5 g, 4.0 mmol) in 30 mL of EtOH was stirred and refluxed for 20 h. The precipitate formed was filtered and washed with hot EtOH, yielding the compound **2** (1.09 g, light yellow solid, 87.7 %); mp 165-167 °C; ¹H NMR (400 MHz, DMSO-*d*₆): 7.98 (s, 1H), 6.56 (s, 1H), 4.79 (br, 3H), 3.55 (s, 6H), 3.14 (m, 4H), 2.50 (m, 4H), 1.80 (m, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 167.9, 160.3, 147.2, 129.7, 111.3, 107.8, 106.5, 64.8, 61.8, 49.8, 49.5, 27.3, 22.4, 21.4, 20.6. *m/z* 389.3.

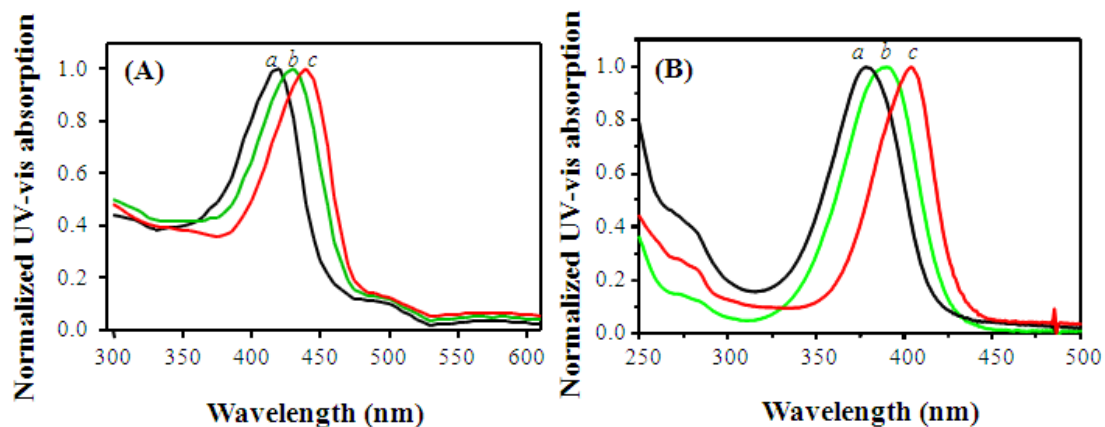


Figure S1. Normalized UV-vis absorption spectra of; coumarin-derivative **1** dye ($2\ \mu\text{M}$) (A) and coumarin-derivative dye **2** ($4\ \mu\text{M}$) (B) in ethanol (*a*), in aqueous solution (*b*) and with CB7 ($100\ \mu\text{M}$) in aqueous solution (*c*).

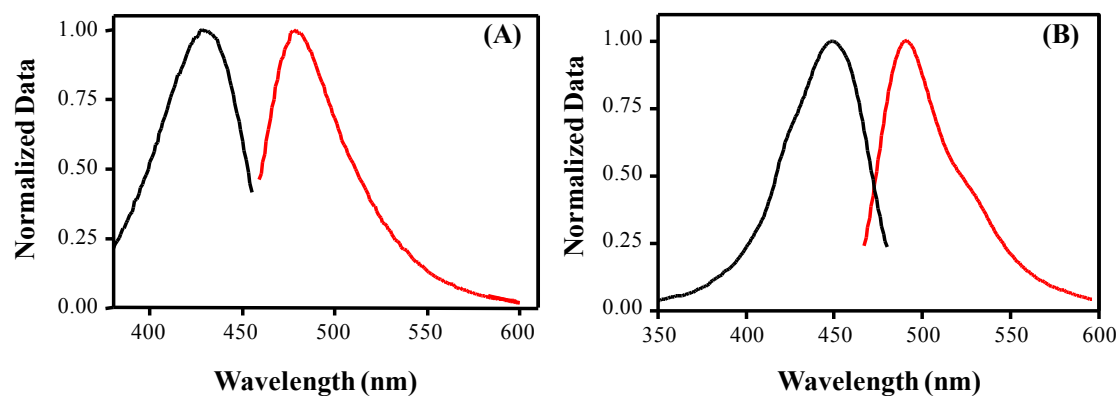


Figure S2. Normalized excitation and emission spectra of coumarin-derivative dyes: **1** ($2\ \mu\text{M}$) (A) and **2** ($4\ \mu\text{M}$) (B), excitation and emission slits of $5\ \text{nm}$ were used. All spectra were assessed in aqueous solution.

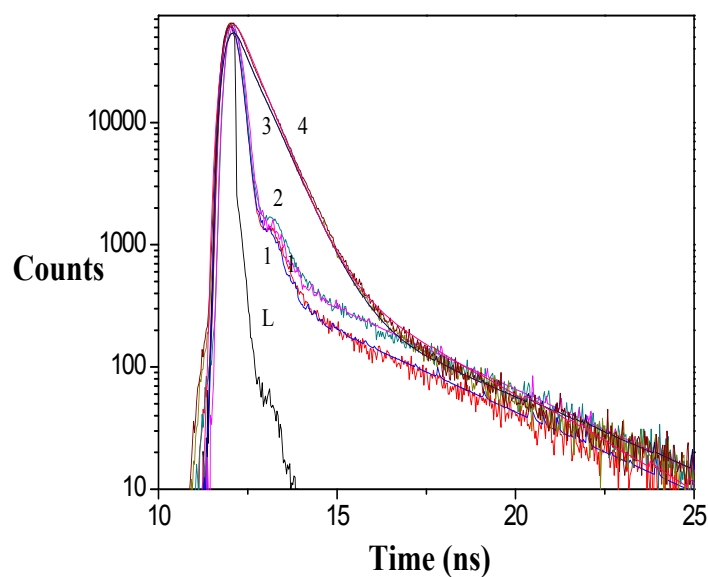


Figure S3. Time-resolved fluorescence decay profiles ($\lambda_{\text{ex}}=405\text{nm}$, $\lambda_{\text{em}}=480\text{ nm}$) of the dye **1** ($5\ \mu\text{M}$) at pH=5.0 (1) and at pH=2.0 (2); the **1**-CB7 complex at pH=5.0 (3) and at pH=2.0 (4). L corresponds at the instrument response function (IRF). The fitted (solid lines) values are presented in the Table 1.

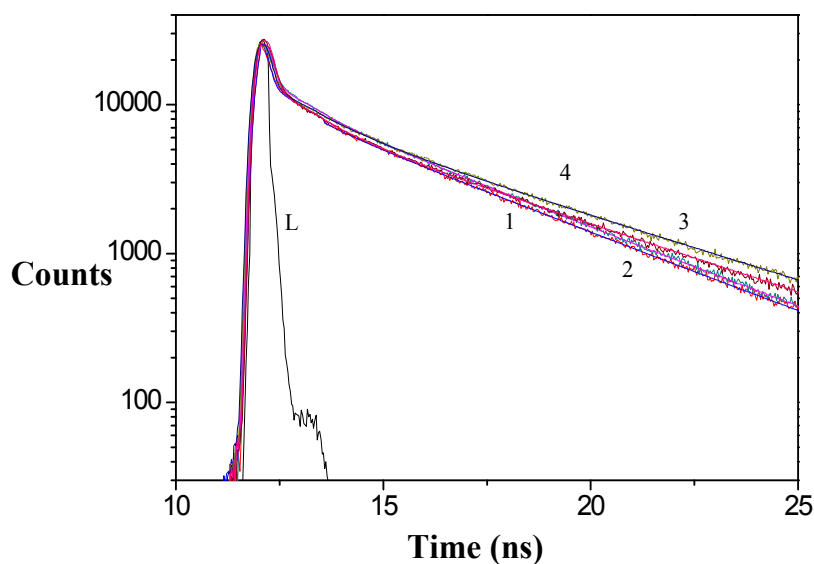


Figure S4. Time-resolved fluorescence decay profiles ($\lambda_{\text{ex}}=405\text{nm}$, $\lambda_{\text{em}}=490\text{ nm}$) of the dye **2** ($5\ \mu\text{M}$) at pH=5.0 (1) and at pH=2.0 (2); the **2**-CB7 complex at pH=5.0 (3) and at pH=2.0 (4). L corresponds at the instrument response function (IRF). The fitted (solid lines) values are presented in the Table 1.

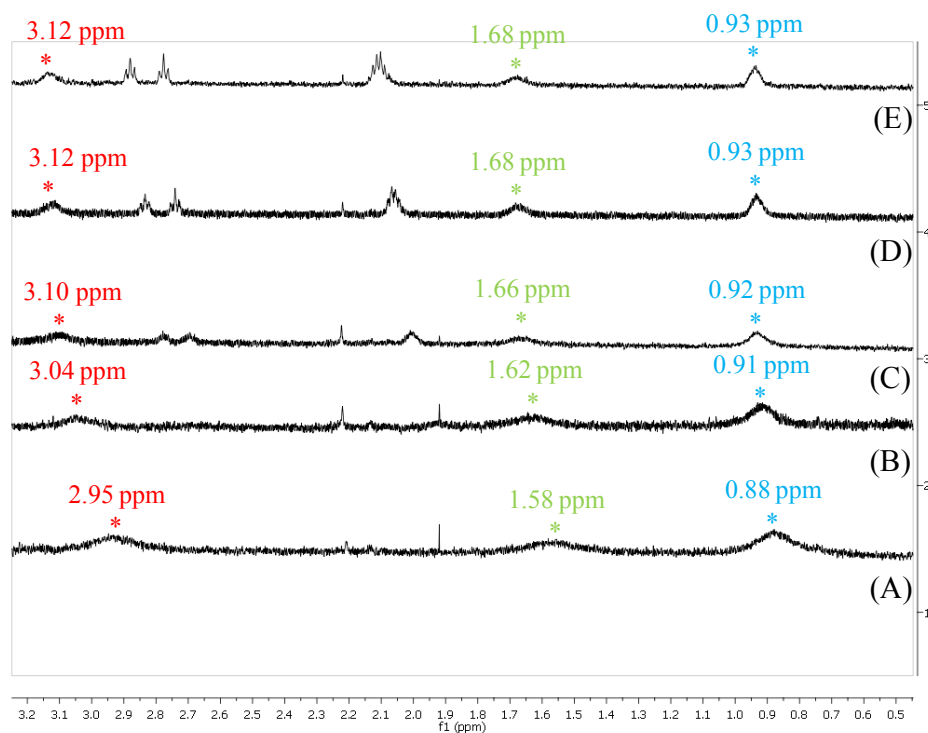


Figure S5. ^1H -NMR titration of TPA-CB7 complex (0.15 mM) with dye **2** (in DCI (10%)– D_2O); (A) no dye **2**, (B) 0.5 eq., (C) 1.0 eq., (D) 2.0 eq. and (E) 3.0 eq.

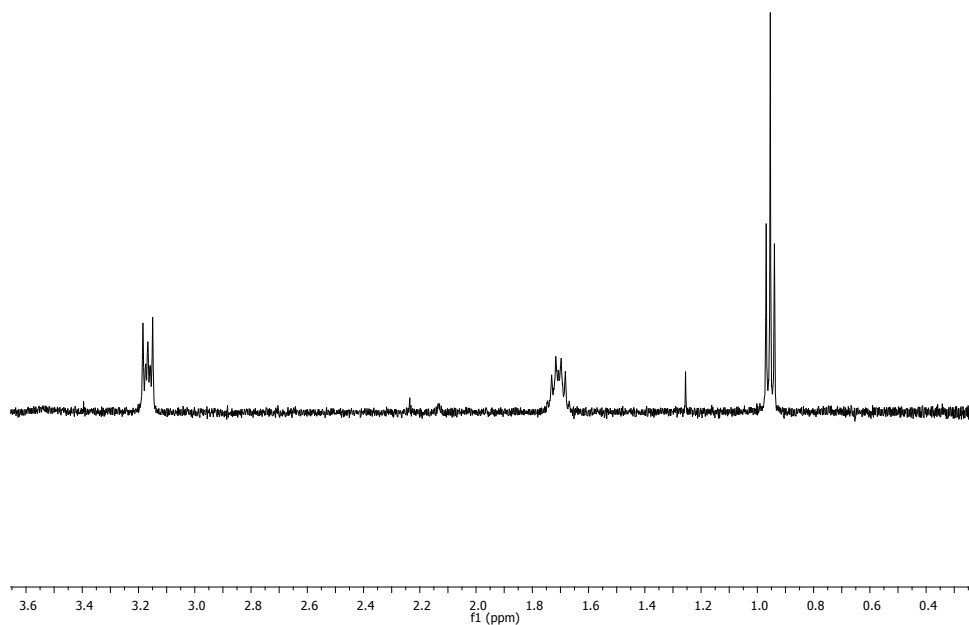


Figure S6. Partial ^1H NMR spectra (750 MHz) for tetrapropylammonium bromide (TPA) 0.15 mM in DCI (10%)- D_2O .

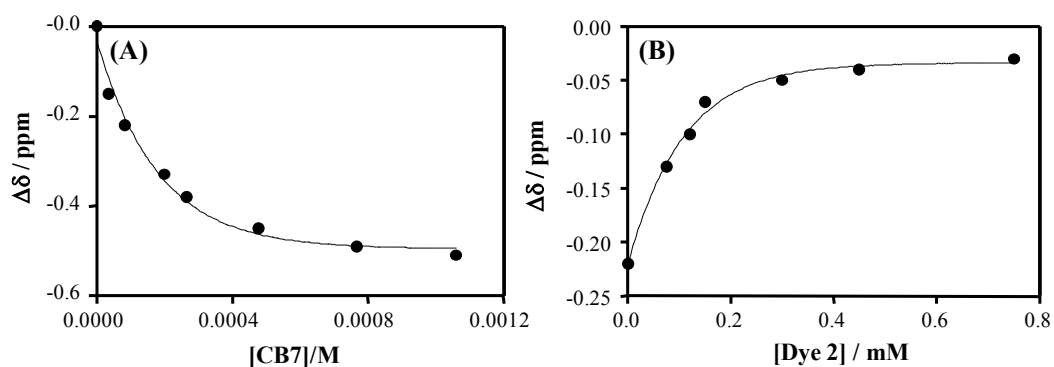


Figure S7. ^1H NMR titration experiments of the dye **2** using competitive displacement of tetrapropylammonium bromide (TPA) from CB7. (A) ^1H NMR titration curve for the binding of TPA (0.15 mM) with increasing concentrations of CB7. (B) Competitive ^1H NMR titration of a solution containing TPA (0.15 mM) plus CB7 (0.15 mM) with increasing concentrations of Dye **2**.

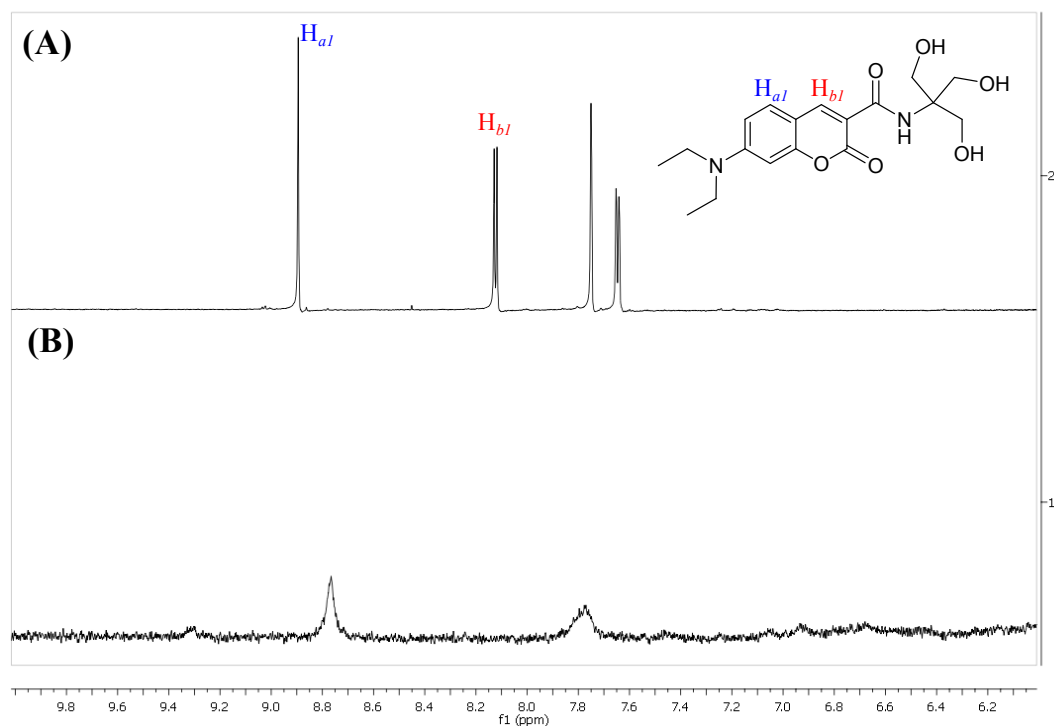


Figure S8. Partial ^1H NMR spectra (750 MHz) comparison for: (A) **1** alone in DCl (10%)- D_2O and (B) **1** and CB7 (3 eq.).

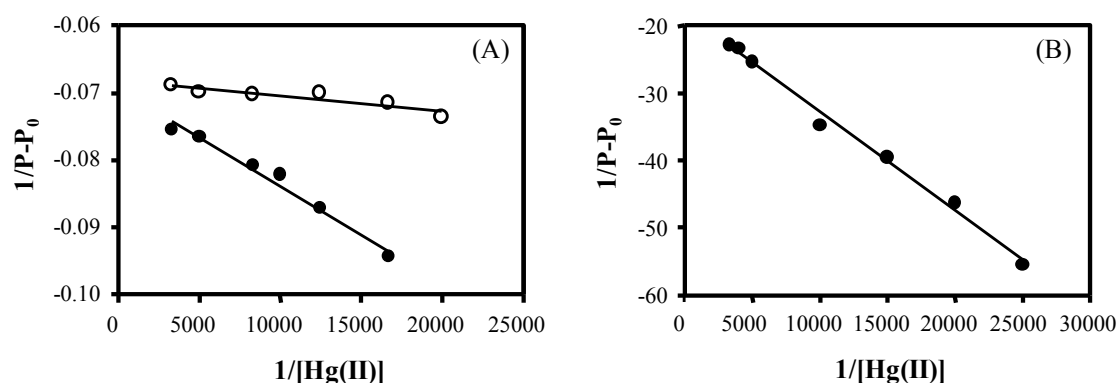


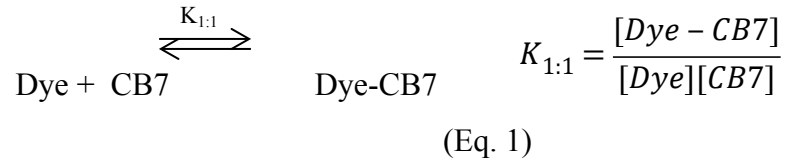
Figure S9. Benesi-Hildebrand plots for dye **1** by fluorescence emission (A) and dye **2** using UV/Vis absorbance (B) with added Hg^{2+} . The symbols represents: (\circ) solutions containing the dye in the absence of CB7 and (\bullet) in the presence of CB7 (both at pH = 2).

Table S1. Apparent binding constants for mercuric ions binding to coumarin-derivative dyes alone and complexed by cucurbit[7]uril.

Experimental Conditions		Apparent Binding Constant (K_b , M^{-1})
1+Hg(II)	(pH=2.0)	$(4.3 \pm 0.9) \times 10^4$
1+Hg(II)+CB7	(pH=2.0)	$(1.6 \pm 0.6) \times 10^7$
1+Hg(II)	(pH=5.0)	$(1.9 \pm 0.2) \times 10^4$
1+Hg(II)+CB7	(pH=5.0)	$(2.1 \pm 0.4) \times 10^4$
2+Hg(II)	(pH=2.0)	$(1.2 \pm 0.5) \times 10^4$
2+Hg(II)+CB7	(pH=2.0)	$(1.6 \pm 0.2) \times 10^4$
2+Hg(II)	(pH=5.0)	$(1.1 \pm 0.4) \times 10^4$
2+Hg(II) + CB7	(pH=5.0)	$(1.5 \pm 0.4) \times 10^4$

Determination of apparent binding constants for the 1:1 complex.

The model used is based on the assumption that a 1:1 complex is formed between each dye and CB7,⁴



The following equation (Eq. 2) for the fluorescence intensity change with CB7 concentration can be derived, when CB7 is in excess respect to dye.^{5,6}

$$I_f = \frac{I_f^0 + I_1 K_{1:1} [\text{CB7}]_0}{1 + K_{1:1} [\text{CB7}]_0} \quad (\text{Eq. 2})$$

where I_f^0 is the fluorescence intensity of dye without CB7, I_1 is the fluorescence intensity from the 1:1 complex when all dye molecules form complexes with CB7, $[\text{CB7}]_0$ is the initial concentration of CB7, and $K_{1:1}$ is the equilibrium constant for the complex formation (Eq. 1). Using a nonlinear regression method, the fluorescence enhancement data are directly fitted with the above equation and the equilibrium constant is estimated. However, when the concentration of CB7 is not in excess respect to the dye, other equations must be considered (Eq. 4-7). It is important to note that the fitting for 1:1 model complexation has been described in further detail by Nau *et al.*⁷

As recently reported by Barooah *et al.* (2014),⁸ for 7-diethylamino group-containing coumarin derivative, the observed emission intensity (I_f) at any specific wavelength is expressed as an average of all the fluorescent species in the medium, that can be described as:

$$I_f = I_0^f \frac{[\text{dye}]_{eq}}{[\text{dye}]_0} + I_{\text{CB7-dye}} \frac{[\text{dye} - \text{CB7}]_{eq}}{[\text{dye}]_0} \quad (\text{Eq. 3})$$

where, I_0^f is the fluorescence intensity of the dye in the absence of CB7 and $I_{\text{dye-CB7}}$ are the fluorescence intensity of the corresponding 1:1 complex with CB7 in solution. The I_f (Eq. 3) can be expressed in terms of binding constant (Eq. 4):

$$I_f = \frac{I_f^0 + I_1 K_{1:1} [CB7]_f}{1 + K_{1:1} [CB7]_f} \quad (\text{Eq. 4})$$

To solve equation (Eq. 4) it is necessary to know the concentration of uncomplexed CB7 ($[CB7]_f$). This concentration can be obtained by means a simulation procedure, supposing that the complexes formed with the CB7 has a stoichiometric ratio 1:1 (as above mentioned). The complexation constant of the dye by CB7 is expressed as equation 1. The mass balance for the total concentrations of dye and CB7 are given by:

$$[Dye]_T = [Dye]_f + [Dye-CB7] \quad (\text{Eq. 5})$$

$$[CB7]_T = [CB7]_f + [Dye-CB7] \quad (\text{Eq. 6})$$

The combination of these equations with the binbing constant gives a second order equation for the concentration of uncomplexed CB7 (Eq. 7):

$$a [CB7]_f^2 + b [CB7]_f + c = 0 \quad (\text{Eq. 7})$$

Where,

$$a = K_{1:1}$$

$$b = K_{1:1} [S]_T - K_{1:1} [CB7]_T + 1$$

$$c = -[CB7]_T$$

The equation (7) was solved for different values of $K_{1:1}$, in order to obtain $[CB7]_f$. The value of $K_{1:1}$ is those for which we obtain the best root mean-square deviation values in the fitting of equation Eq. 4.

On the other hand, the influence of CB7 on the ^1H chemical shifts of TPA was studied in the presence of dye **2** (Figure S5). Equation (8) represents the mathematical model⁹ based on a 1:1 host-guest complex and the experimental points used are obtained from results presented in Figure S5.

$$\Delta\delta = \frac{\Delta\delta_{1:1} K_{1:1} [CB7]_f}{1 + K_{1:1} [CB7]_f}$$

(Eq. 8)

The combination of this equation with the binbing constant gives a third order equation for the concentration of uncomplexed CB7 (Eq. 9):

$$a [CB7]_f^3 + b [CB7]_f^2 + c [CB7]_f + d = 0 \quad (\text{Eq. 9})$$

Where,

$$a = K_{1:1}K_{2CB7}$$

$$b = K_{1:1} + K_{2CB7} + K_{1:1}K_{2CB7}([TPA] + [2] - [CB7])$$

$$c = 1 + K_{1:1}([TPA] - [CB7]) + K_{2CB7}([2] - [CB7])$$

$$d = -[CB7]_T$$

The equation (9) was solved for different values of $K_{1:1}$, in order to obtain $[CB7]_f$. The value of $K_{1:1}$ is those for which we obtain the best root mean-square deviation values in the fitting of equation Eq. 8. The detail for the fitting of a competitive binding model is described by Nau *et al.*¹⁰

References

1. Y. Ma, W. Luo, P. J. Quinn, Z. Liu, R. C. Hider, *J. Med. Chem.*, 2004, **47**, 6349.
2. J. Yao, W. Dou, W. Qin, W. Liu, *Inorg. Chem. Comm.* 2009, **12**, 116.
3. O. García-Beltrán, N. Mena, O. Yañez, J. Caballero, V. Vargas, M. Nuñez, B. Cassels, *Eur. J. Med. Chem.* 2013, **67**, 60.
4. M. Pessêgo, J. A. Moreira, L. Garcia-Rio, *Chem. Eur. J.* 2012, 7931.
5. A. V. Deshpande, L. V. Jathar, J. R. Rane, *J. Fluoresc.* 2009, **19**, 607.
6. A. Muñoz de la Peña, F. Salinas, M. J. Gómez, M. I. Acedo, M. J. Sánchez Peña, *Incl. Phenom. Mol. Rec. Chem.* 1993, **15**, 131.
7. (a) H. Bakirci, X. Zhang, W. M. Nau, *IJ. Org. Chem.* 2005, **70**, 39. (b) W. M. Nau, X. Zhang, *J. Am. Chem. Soc.* 1999, **121**, 8022.
8. N. Barooah, M. Sundararajan, J. Mohanty, A. C. Bhasikuttan, *J. Phys. Chem. B* 2014, **118**, 7136.
9. K. A. Connors, *Binding Constants: The Measurement of Molecular Complex Stability*, Wiley, New York, 1987, pp. 189-215.
10. (a) A. Hennig, H. Bakirci, W. M. Nau, *Nature Methods* 2007, **4**, 629. (b) M. Bailey, A. Hennig, V. D. Uzunova, W. M. Nau, *Chem. Eur. J.* 2008, **14**, 6069.