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ORIGINAL ARTICLE

Fluorescent cyclodextrins bearing metal binding sites and their use for chemo- and enantioselective sensing of amino acid derivatives

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Abstract Ditopic receptors based on cyclodextrins bearing a metal binding site were used as enantioselective fluorescence sensors, which were able to generate different responses in the presence of D- or L-amino acids. The performances of the selectors as a function of their structure were evaluated, and the same analysis was extended to other analytes. In this work, this approach is used for the enantiomers of a series of amino acid derivatives and in particular of 2-aminocaprolactam. The results showed that the ability of these sensors to perform enantiomeric analysis can be extended to other analytes of interest in organic synthesis such as amino acid amides and α-aminolactams.

Keywords Fluorescent sensors · Enantioselectivity · Amino acids derivatives · Cyclodextrins

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Introduction

The design of optical sensors and in particular fluorescent sensors is a very active field of research, due to their potential use in devices for on-line monitoring and fast screening [1]. Enantioselectivity in this context is a property highly desirable, since the enantiomers of many compounds differ in their biological properties [2–4].

Cyclodextrins bearing a metal binding site can be used as ditopic receptors able to combine the coordination properties of a metal ion and recognition of apolar moieties by the cyclodextrin cavity [5, 6]. In our group, this type of receptors has been used in combination with fluorogenic groups to generate fluorescent cyclodextrins able to produce a fluorescent signal upon binding to metal ions or organic molecules [7, 8].

Copper(II) complexes with chiral ligands have been used for many years as enantioselective selectors in ligand exchange chromatography [9]. Using a similar approach, we have recently focused our attention on the development of enantioselective fluorescence sensors based on the fluorescent cyclodextrins reported in Fig. 1, which were able to generate different responses in the presence of D- or L- amino acids [10, 11]. The performances of the selectors as a function of their structure were evaluated, and the same analysis was extended to other analytes [12].

The system was suitable for fast screening of enantiomeric excess using microplate reader and a simple procedure [13]. In this work, we show how this approach can be used for the enantiomeric discrimination of amino acid derivatives and in particular of 2-aminocaprolactam, which is an important industrial intermediate for the synthesis of lysine, and compare the results obtained with those of free amino acids.

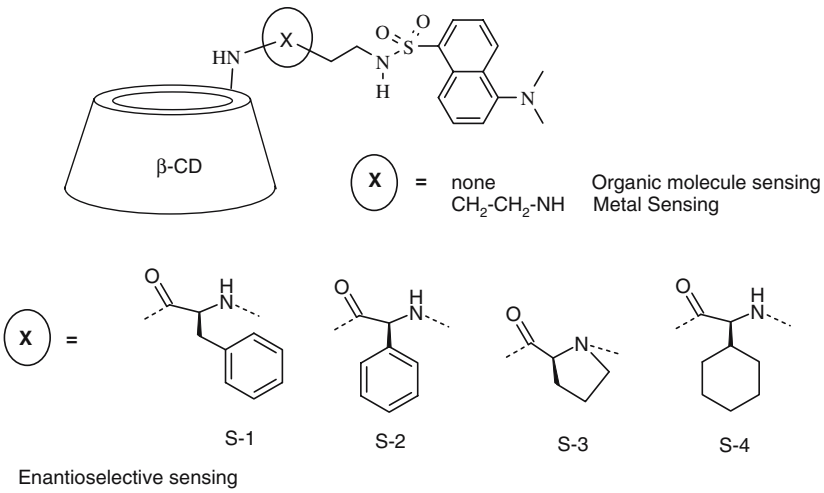

Fig. 1 Structure and characteristics of the cyclodextrins **S-1**, **S-2**, **S-3**, and **S-4** used in the present study

Experimental

Materials

Starting materials were obtained from commercial suppliers and used without further purification. Cyclodextrins **S-1-4** were synthesized as described previously [11, 12].

Stock solutions of D- or L-valine and D- or L-proline and analytes **1–10** copper(II) 2:1 complexes were prepared in water by weighing the exact amount of the amino acid, adding the corresponding volume of a $CuSO_4$ $5H_2O$ (20 mM), and then diluting to the final volume (10 ml) with water. Under these conditions, the 2:1 amino acid:copper(II) complex is completely formed, according to the stability constants. Solutions of different enantiomeric excess were prepared by mixing appropriate volumes of these solutions. Stock solutions of cyclodextrins **S-1**, **S-2**, and **S-3** (0.7–1.2 mM) were prepared in double distilled water. Measuring solutions (60 μM) were prepared by dilution of these concentrated solutions in 0.1 M sodium borate buffer at pH = 7.0.

For derivatives **11–14**, a stock solution of the analyte in DMSO was prepared. To a solution of cyclodextrin **S-3** (60 μM), copper(II) in equimolar amount and then a 2:1 excess of the analyte were added.

For the calibration curves reported in Fig. 4 solutions of 2-aminocaprolactam (12 mM) and copper (II) (6 mM) at different enantiomeric composition were obtained by mixing appropriate amounts of the stock solutions. A solution of cyclodextrin **S-2** (60 μM) in 0.1 M borate buffer was prepared. 500 μl of these solutions were added to 30 μl of the 2-aminocaprolactam / copper(II) solutions (2-aminocaprolactam / copper(II)/cyclodextrin molar ratio = 12:6:1).

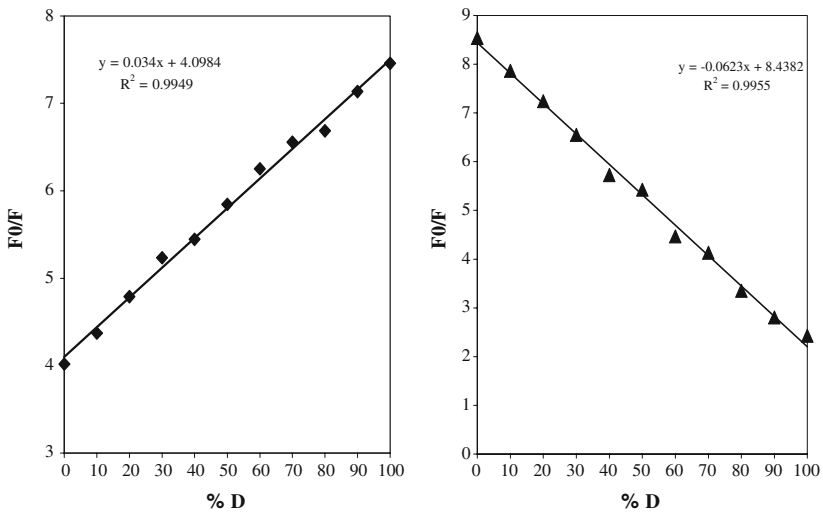

Fig. 2 Calibration curves obtained by quenching method for (**a**) valine and (**b**) proline using a 1 μM solution of cyclodextrin **S-4** upon addition of a 10-fold excess of $Cu(AA)_2$ complex in borate buffer (0.1 M) at pH = 7

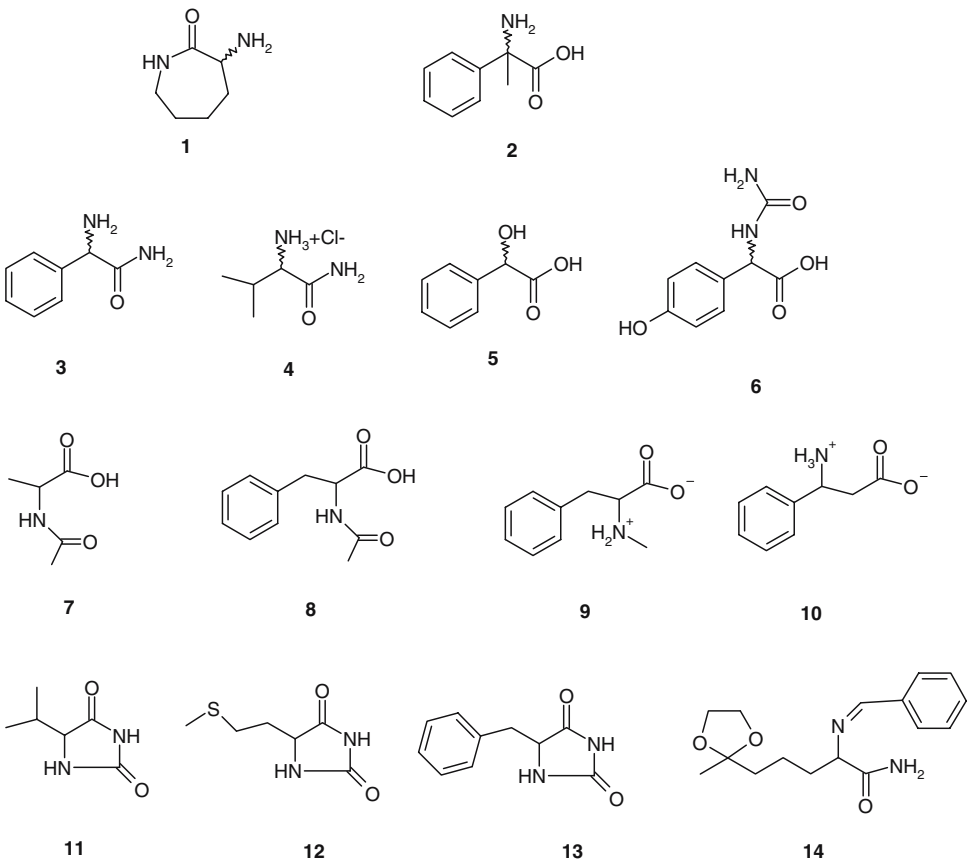

Fig. 3 Structure of the analytes **1–14** used in the present study

Apparatus

Fluorescence spectra were recorded on a Perkin Elmer Ls50B instrument in a 1×0.2 cm quartz cell thermostated at 25°C. In the calibration experiments reported in Fig. 2, 0.5 ml of the 60 μM solution of cyclodextrin (**S-4**) were titrated in the cell by adding 6×10^{-3} M $Cu(Val)_2$ or $Cu(Pro)_2$ of different enantiomeric composition in water with a 10 μl syringe.

Procedures

Three measurements were performed for each solution. Fluorescence intensities at the maximum emission wavelength were used; correction of the fluorescence intensity of all the samples was made according to the expression $I_n=I/I_{st}$, where I is the observed fluorescence intensity and I_{st} is the intensity of the reference solution of the free cyclodextrin, both measured at the same excitation and emission wavelength.

Results and discussion

The modified cyclodextrins bearing a fluorophore linked to the upper rim (C6 atom of one glucose unit), reported in Fig. 1, are highly fluorescent, if compared with the free fluorophore in solution [11, 12], due to the self-inclusion of the dansyl moiety within the cyclodextrin cavity. Since donor atoms are present in the linking moiety connecting the cyclodextrin to the dansyl group, they are able to interact with copper(II) ions giving rise to a non-fluorescent binary complex.

When an amino acid is added to this complex, a ternary complex is formed, whose stability is depen-

Table 1 Comparison of enantioselectivities in fluorescence intensities (and relative standard deviations) obtained by sensor **S-1, S-2** and **S-3** upon addition of the copper(II) complex of 2-aminocaprolactam. Fluorescence intensities are normalized to free cyclodextrin

CD	$F_{L(S)}$	$F_{D(R)}$	sd (L)	sd (D)	*t*-value	Significance	F_D/F_L
S-1	0.073	0.097	0.007	0.002	–6.593	>99	1.33
S-2	0.047	0.067	0.001	0.001	–28.284	>99	1.43
S-3	0.0132	0.0123	0.0008	0.0004	1.743	>80	0.93

dent on the type of amino acid and to its absolute configuration [11].

In order to exploit the enantioselectivity in the formation of these ternary complexes for obtaining an enantioselective signal, it was found that a fluorimetric titration of the free cyclodextrin with the amino acid copper(II) complex can be used, according to the following scheme:

$$\mathrm{Cu(L\text{-}AA)_2} + \mathrm{CD\ (fluorescent)} \overset{K_L}{\leftrightarrows} \mathrm{L\text{-}AA} + \mathrm{Cu(CD)(L\text{-}AA)(non\ fluorescent)} \quad (1)$$

$$\mathrm{Cu(D\text{-}AA)_2} + \mathrm{CD\ (fluorescent)} \overset{K_D}{\leftrightarrows} \mathrm{D\text{-}AA} + \mathrm{Cu(CD)(D\text{-}AA)(non\ fluorescent)} \quad (2)$$

Where L-AA and D-AA are the D- and L-amino acid (in the anionic form), CD is the cyclodextrin sensor, and $Cu(L\text{-}AA)_2$ or $Cu(D\text{-}AA)_2$ are the binary copper(II) complexes and Cu(CD)(L-AA) and Cu(CD)(D-AA) are the ternary copper(II)/cyclodextrin/amino acid complexes.

Since the two constants for the formation of diastereomeric ternary complexes K_L and K_D are in principle different, the amount of free CD can be different when the two enantiomers of the same amino acid are used. We have previously demonstrated that using a sufficiently high concentration of the titrating complex, the fluorescence response depends mainly on the enantiomer used, while the effect of the amino acid concentration is negligible [13]. A systematic study showed that cyclodextrins bearing a L-amino acid derivative as linker (**S-1-4**) gave the best enantioselectivity [11, 12]. Using the cyclodextrin **S-4**, which showed good fluorescence response and enantioselectivity, it was possible to perform calibration experiments at very low ligand concentration (1 μM); fluorimetric titrations of the cyclodextrin **S-4** with copper(II) complexes of proline and valine as a function of their enantiomeric excess were performed (Fig. 2).

Since these experiments are based on enantioselective fluorescence quenching, the Stern-Volmer equation can be used in the following form [13]:

$$F_0/F = 1 + K_L[Cu(L\text{-}AA)_2] + K_D[Cu(D\text{-}AA)_2] = 1 + [K_L + (K_D - K_L)\, x_D]\,[Cu(AA)_2] \quad (3)$$

where F_0 is the fluorescence intensity of the cyclodextrin solutions without quencher, F is the fluorescence observed after addition of the $Cu(AA)_2$ complex, x_L and x_D are the molar fraction of the two enantiomers and K_D and K_L are the Stern-Volmer constants of the two enantiomers in static quenching (corresponding to the equilibrium constants for the formation of the two ternary complexes). If the total amino acid and copper concentration and hence that of the $Cu(AA)_2$ complex is kept constant in the experiment, F_0/F varies linearly with the enantiomer molar fraction x_D. This compound, containing a rigid and sterically demanding cyclohexyl group, was found to be able to give good enantioselectivity, though it was not tested previously in titration experiments. These data further increase the applicability of this test to fast sensing schemes, since only a very small amount of the selector (10^{-9} mol) was used for a single set of measurements. The enantioselectivity was inverted for the two amino acids, in agreement with the enantioselectivity observed for this type of selectors [13].

This approach can be useful when the pure amino acids are present, since a mixture of different amino acids would give rise to a combination of effects. However, if this system is used for the measurement of the enantioselectivity of the reactions producing only one compound, it can allow to have a rapid screening of reaction conditions. In this context, we evaluated the chemo- and enantioselectivity of the response towards different amino acid derivatives, which could be

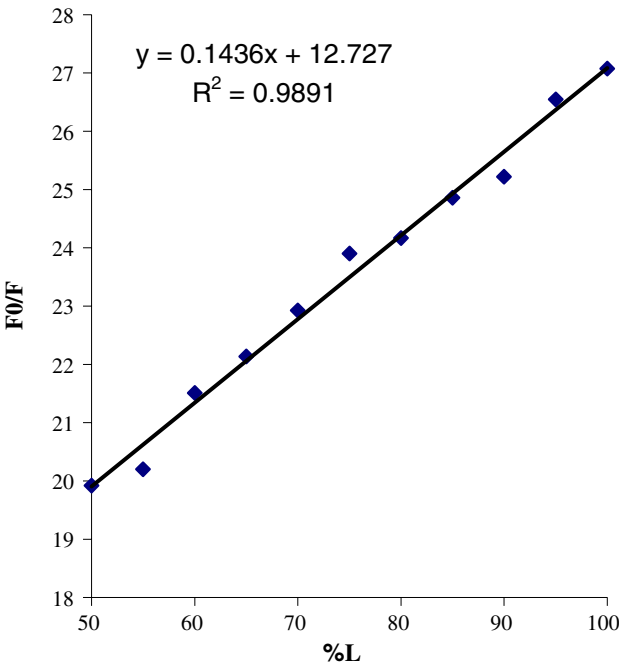

Fig. 4 Calibration curve obtained by quenching method for 2-aminocaprolactam (**1**) using a 60 μM solution of cyclodextrin **S-2** upon addition of a 6-fold excess of $Cu(\mathbf{1})_2$ complex in borate buffer (0.1 M) at pH = 7

Table 2 Comparison of enantioselectivities in fluorescence intensities (and relative standard deviations) obtained by sensor **S-2** upon addition of the copper(II) complexes of the amino acids Val and Pro and of analytes **1–15** in a 10-fold excess. Fluorescence intensity are normalized to free **S-2**. Analytes 11–15 were tested using a 2:1 excess in borate buffer in water: DMSO 9:1

Analyte	$F_{L(S)}$	$F_{D(R)}$	sd (L)	sd (D)	*t*-value	Significance	F_L/F_D
Val	0.26	0.296	0.010	0.003	38,157	>99	1.78
Pro	0.286	0.503	0.028	0.013	-12,175	>99	0.56
2	0.405	0.222	0.040	0.015	12,851	>99	1.82
3	0.110	0.085	0.021	0.004	3,308	>99	1.29
4	0.081	0.063	0.005	0.006	3,992	>99	1.29
5	0.163	0.159	0.006	0.004	1,240	>60	1.03
6	0.122	0.115	0.022	0.007	0.678	>40	1.06
7	0.211	0.214	0.002	0.002	–1.84	>80	0.99
8	0.100	0.099	0.003	0.010	0.166	<20	1.010
9	0.330	0.162	0.011	0.005	24.082	>99	2.037
10	0.088	0.082	0.002	0.003	2.882	>90	1.073
11	1.00	1.01	0.10	0.11	–0.117	<20	1.01
12	1.04	1.04	0.04	0.03	0.000	<20	1.00
13	1.04	1.03	0.09	0.09	0.136	<20	0.99
14	0.99	1.11	0.13	0.04	–0.528	>60	1.12

intermediate for synthetic pathways of industrial interest, and then their enantioselectivity (Fig. 3)

We therefore chose to explore the possibility to use these cyclodextrins for discriminating the enantiomers of 2-aminocaprolactam (**1**, Fig. 3), which is an important amide intermediate for the synthesis of lysine, and it can be processed by very enantiospecific enzymes.

High quenching efficiency was obtained for both enantiomers of this analyte, suggesting that the ternary complex formed is of high stability (Table 1).

The best performing structure in terms of enantioselectivity was in this test the cyclodextrin **2**, containing a L-phenylglycine synthon in the side arm. We therefore tested this cyclodextrin in a calibration using different enantiomeric composition (in the range 50–100% L) of this analyte and cyclodextrin **S-2** as enantioselective sensor (Fig. 4).

A linear correlation was found also in this case; however, due to the low enantioselectivity, the correlation coefficient was lower than for the above reported amino acid curves. However, this method could be used to at least a rapid screen for discriminating between highly or low enantioselective reactions.

The same cyclodextrin (**S-2**) was then used as a selector for the other analytes, with the same approach. Amino acids were found to be best recognized in terms of both response and enantioselectivity. The enantioselectivities in fluorescence response, together with the statistical analysis are reported in Table 2.

Good enantioselectivity was observed for *N*-alkylated amino acids and for the amino acid amides, while other derivatives such as *N*-acetyl or *N*-carbamoyl amino acids and hydantoines were practically uneffective under the same conditions. A common feature of the species discriminated is their ability to bind copper (II) under the conditions used (pH = 7).

The low enantioselectivity observed in the case of 2-aminocaprolactam (compared to amino acids) could be due to the particular geometry of this amino acid amide. Primary amino acid amides can chelate copper(II) at mildly basic pH through the coordination of the amino group and of the deprotonated amide nitrogen [14]. In the case of the ternary complexes formed with the cyclodextrin derivatives, both *cis* and *trans* species coordinated through the amide carbonyl groups could be present (Fig. 5), thus reducing the enantioselectivity of their formation, which requires a definite preferential geometry, such as that observed for the amino acid ternary complexes.

Conclusion

The present work completes the overall information on the possibility to use the fluorescent cyclodextrins reported in Fig. 1 as tools for the development of fast sensing methods. The first case of enantiomeric discrimination of 2-aminocaprolactam by fluorescence sensor, together with the results obtained with other amino acid amides, suggest that the this approach can

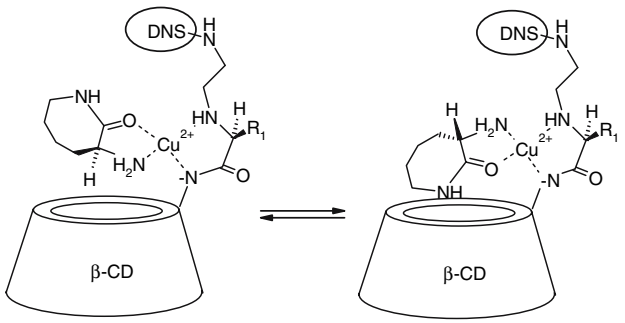

Fig. 5 Proposed *cis-trans* equilibrium of the cyclodextrin/copper(II)/2-caprolactam complex

be extended beyond the amino acid model previously proposed.

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