Enantioselective Fluorescence Sensing of Amino Acids by Modified Cyclodextrins: Role of the Cavity and Sensing Mechanism

Sara Pagliari,[a, c] Roberto Corradini,*[a] Gianni Galaverna,[a] Stefano Sforza,[a] Arnaldo Dossena,[a] Marco Montalti,[b] Luca Prodi,[b] Nelsi Zaccheroni,[b] and Rosangela Marchelli[a]

Abstract: Two selectors based on modified cyclodextrins containing a metal binding site and a dansyl fluorophore—6-deoxy-6-*N*-(N^{α}-[(5-dimethylamino-1-naphthalenesulfonyl)aminoethyl]phenylalanylamino-β-cyclodextrin—containing D-Phe (**3**) and L-Phe (**4**) moieties were synthesized. The conformations of the two selectors were studied by circular dichroism, two-dimensional NMR spectroscopy and time-resolved fluorescence spectroscopy. Cyclodextrin **4** was found to have a predominant conformation in which the dansyl group is self-included in the cyclodextrin cavity, while **3** showed a larger proportion of the conformation with the dansyl group outside the cavity. As a consequence, the two cyclodextrins were found to bind copper(II) with different affinities, as revealed by fluorescence quenching in competitive binding measurements. Addition of D- or L-amino acids induced increases in fluorescence intensity, which were dependent on the amino acid used and in some cases on its absolute configuration. The cyclodextrin **4** was found to be more enantioselective than **3**, suggesting that the self-inclusion in the cyclodextrin cavity strongly increases the chiral discrimination ability of the copper(II) complex. Accordingly, a linear fluorescent ligand N^{α}-[(5-dimethylamino-1-naphthalenesulfonyl)aminoethyl]-N^{1}-propyl-phenylalaninamide, which has the same binding site and absolute configuration as **4**, showed very low chiral discrimination ability. The enantioselectivity in fluorescence response was found to be due to the formation of diastereomeric ternary complexes, which were detected by ESI-MS and by circular dichroism. Time-resolved fluorescence studies showed that the fluorescence of the dansyl group was completely quenched in the ternary complexes formed, and that the residual fluorescence was due to uncomplexed ligand.

Keywords: amino acids · copper · cyclodextrins · enantioselectivity · sensors

Introduction

Optical sensing by molecular recognition principles has become increasingly important in recent years.[1] Optical sensing molecules have been designed for ions and organic compounds, by use of supramolecular chemistry in combination with optical and photophysical properties.[2] Fluorescent sensors are of particular interest, due to the high sensitivity and selectivity of the detection method,[3] and to their properties as photoionic gates and switches.[4] Fluorescent sensing molecules capable of detecting metal ions have been reported, based on changes in fluorescence intensity due to photoinduced electron transfer (PET)[5] or proton transfer processes,[6] or shifts in the fluorescence maxima induced by complexation,[7] by excimer formation[8] or by irreversible reactions.[9] Sensors for anions[10] and bioactive organic molecules[11] have also been synthesized, some of them showing remarkable selectivity.

Application of chemosensors as components of an "artificial tongue" for the parallel detection of organic molecules

[a] Dr. S. Pagliari, Prof. R. Corradini, Dr. G. Galaverna, Dr. S. Sforza, Prof. A. Dossena, Prof. R. Marchelli
Dipartimento di Chimica Organica e Industriale
Università di Parma, Parco Area delle Scienze 17/A
43100 Parma (Italy)
Fax: (+39) 0521-905472
E-mail: roberto.corradini@unipr.it

[b] Dr. M. Montalti, Dr. L. Prodi, Dr. N. Zaccheroni
Dipartimento di Chimica "G. Ciamician"
Università di Bologna, Via Selmi 2
40126 Bologna (Italy)

[c] Dr. S. Pagliari
Present address:
Cyanagen S.r.l. via Stradelli Guelfi 40/c
40138 Bologna (Italy)

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author. ESI-MS spectra of the ternary mixtures containing cyclodextrin **4**, copper(II) and D- or L-proline.

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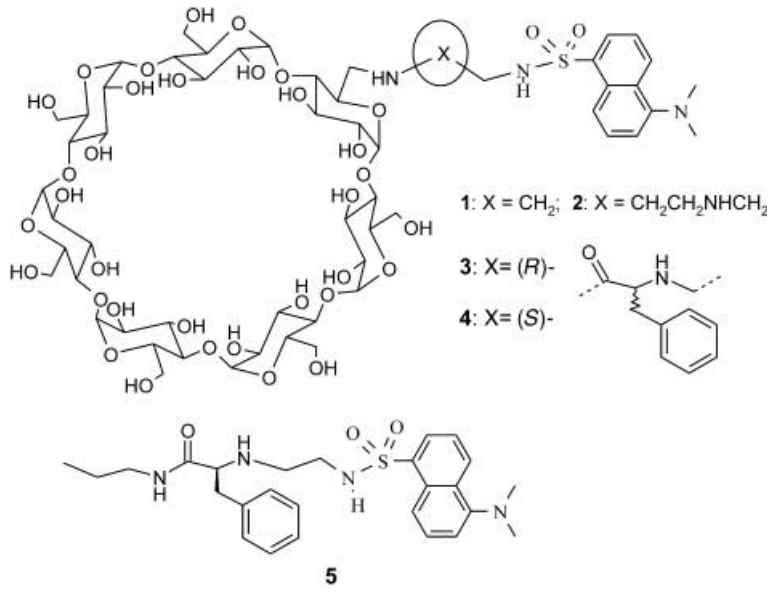
in solution has recently been proposed.[12] One of the essential features of natural taste sensory systems is the ability to discriminate between enantiomers; for example, L-amino acids are bitter, while D-amino acids are sweet. Moreover, enantioselectivity is also one of the most important goals for the sensing of organic substances,[13] since biological activity is strictly correlated with stereochemistry. Although some enantioselective sensors based on enzymes,[14] on piezoelectric effects,[15] or on colorimetric methods[16] have been proposed in recent years, very few examples of enantioselective fluorescence sensors have been reported.[17,18]



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Modified cyclodextrins bearing fluorescent moieties have been used by Ueno and co-workers as sensors for a wide variety of organic guests, including chiral molecules.[19] The advantages of the use of cyclodextrin derivatives are: 1) solubility in water, which circumvents problems with highly lipophilic fluorescent groups that can limit application in biological systems, 2) fluorescence enhancement of some fluorophores through inclusion within the cyclodextrin cavity,[20] 3) the presence of a lipophilic cavity which can act as a recognition motif for apolar groups in water,[21] and 4) the use of intramolecular interactions to obtain rigidly preorganized structures from flexible synthons.

The use of cyclodextrins modified with chiral fluorescent side arms has been described. Cyclodextrin mono-derivatives containing either D- or L-dansylleucine were shown to recognize organic guests with both chemo- and diastereoselectivity, the recognition properties being affected by the configuration of the leucine residue.[22] An L-tryptophan-modified β-cyclodextrin was recently described, and showed enantioselective binding of organic guests such as borneol or menthol.[23] However, this type of approach is not suitable for the enantioselective binding of bifunctional polar molecules such as unmodified amino acids.

Modification with groups containing metal binding moieties allows the use of metal complexes of cyclodextrins as binding sites for organic molecules with donor groups.[24] For example, enantiomeric recognition of unmodified amino acids was performed with the copper complex of a histamine-modified β-cyclodextrin and related molecules.[25]

We have recently described the synthesis and binding properties of modified β-cyclodextrins **1** and **2**, containing a binding site for copper(II) and a dansyl fluorophore.[26,27]

The cyclodextrin CD-dien-DNS **2** has been found to undergo fluorescence quenching in the presence of copper(II), thus acting as a fluorescent chemosensor with good metal ion selectivity. One interesting property of the complex Cu-**2** was that the fluorescence was "switched on" in the presence of amino acids, with responses depending on the amino acid used,[27] as a result of the formation of ternary complexes.[28] However, no enantioselectivity was observed in this case.

In a preliminary communication, we reported the use of this approach to obtain enantioselective fluorescence sensors for unmodified amino acids. We synthesized the two modified cyclodextrins **3** and **4**, each bearing an N^{α}-(N^{2}-dansylaminoethyl)-D-(or L)-phenylalanine unit as a copper(II)-binding moiety.[29] Our design being based on our previous experience in enantiomer separation by copper(II) complexes of amino acid amides (ligand-exchange chromatography),[30–31] the fluorescent group was chosen to have an amino, an amide, and a sulfonamide group as binding sites for copper(II), and an additional chiral centre to enhance enantioselectivity. β-Cyclodextrin was chosen as platform, since the size of the cavity is most suited for the complexation of the dansyl residue.[26]

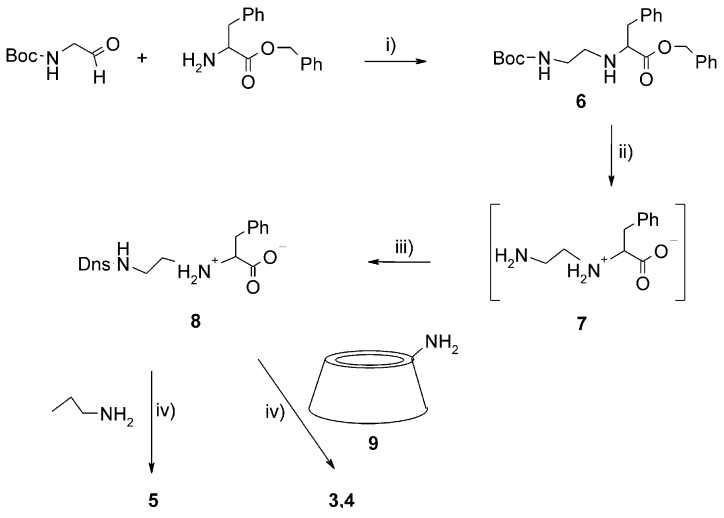
Herein we report a systematic study of the sensing mechanism of the two cyclodextrins **3** and **4** by spectroscopic methods. Comparison between the two cyclodextrins and the analogous compound **5**, without the cyclodextrin part, provided precious insights into the role of the cavity in the recognition process.

The mechanism of fluorescence sensing was studied by steady-state and time-resolved fluorescence and circular dichroism, and the enantioselectivity was evaluated as a function of the analyte amino acid and of the cyclodextrin structure.

Results and Discussion

Synthesis: N^{α}-(N^{2}-Dansylaminoethyl)-D-(or L)-phenylalanine D-**8** or L-**8** were synthesized from D- and L-phenylalanine benzyl ester with a simple two-step strategy (Scheme 1).

tert-Butoxycarbonyl(Boc)-protected aminoethylphenylalanine benzyl ester **6** was first synthesized by a general strategy for the synthesis of chiral peptide nucleic acids



Scheme 1. Synthesis of the cyclodextrins **3** and **4** and of the amide **5**: i) $NaBH_3CN$, CH_3COOH, in MeOH, RT, 2 h; ii) $AlCl_3$, anisole, CH_3NO_2, CH_2Cl_2, 0 °C, 15 min; iii) dansyl chloride, Li_2CO_3 in H_2O/CH_3CN, 0 °C, 4 h; iv) HBTU/DIEA in DMF dry, 3 h.

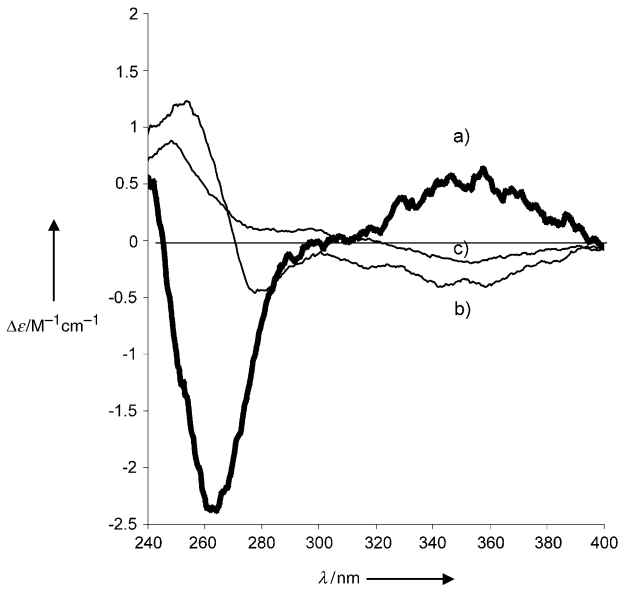


Figure 1. Circular dichroism spectra of: a) **4**, b) **3** in aqueous solution (0.1 M borate buffer, pH 7.3), and c) **5** in MeOH/H_2O 9:1 (0.02 M borate buffer, pH 8.0).

(PNAs).[32] Simultaneous removal of Boc and benzyl groups was carried out with $AlCl_3$. The resulting mixture containing aminoethylphenylalanine (**7**) was treated with dansyl chloride at low temperature (0 °C, in order to avoid sulfonylation of the secondary amine) to yield D-**8** or L-**8.** The two modified cyclodextrins **3** and **4** were synthesized by treatment of 6-deoxy-6-amino-β-cyclodextrin **9** with D-**8** or L-**8**, respectively, in the presence of a coupling reagent (HBTU). Good enantiomeric purities (*ee* = 95% for **3** and 94% for **4**) were obtained, as measured by hydrolysis of the ligands with HCl followed by chiral GC-MS analysis of the resulting *N*-2-aminoethylphenylalanine by a method previously developed by us.[33]

The linear ligand **5** was obtained in a similar way, by treatment of L-**8** with *n*-propylamine.

Conformations of the ligands: The two diastereomeric cyclodextrins **3** and **4** showed different conformational properties in solution. Their circular dichroism spectra, reported in Figure 1, showed 350 nm bands of opposite signs.

In the inclusion complexes of substituted naphthalenes with cyclodextrins, the signs of the induced circular dichroism (ICD) bands are dependent on the type of complex formed (axial or equatorial) and on the position of the chromophore with respect to the cavity (inside or outside).[34] In our previous work we had been able to assign the differences in the ICDs of cyclodextrins **1** and **2** to different orientations of the dansyl moiety within the cavity, as confirmed by NMR data; in particular, an intense negative band in the 350 nm region was associated with deep axial complexation, while a positive one was interpreted as due to equatorial complexation.

In the present case, the positive band of **4** can be interpreted as due to equatorial inclusion of the dansyl group, while the weak negative band of **3** could be due either to axial complexation or to the dansyl group being positioned outside the cavity in an equatorial orientation. The circular dichroism spectrum of **5**, with the same absolute configuration of the side arm of **4**, showed a weak negative nature of the 350 nm band, indicating that the spectrum of the latter compound is not due to the asymmetry of the side arm, but rather to its interaction with the cyclodextrin cavity.

Two-dimensional NMR spectra (ROESY and TOCSY) were used to better characterize the different conformations. Figure 2 reports the portions of the ROESY spectra showing the cross-peaks connecting the aromatic with the cyclodextrin protons.

In the case of the L-Phe-containing cyclodextrin **4**, correlations between the protons of the dansyl group— protons 3′ and 7′ in particular—with those of the cyclodextrin were detected, suggesting equatorial inclusion within the CD cavity. In the case of **3**, only correlations between the phenylalanine aromatic protons and the cyclodextrin cavity were detected, thus suggesting a predominant conformation with the dansyl group outside and the phenyl ring inside or on top of the cavity.

Since the NMR timescale did not allow the contributions of the different conformations to be evaluated, steady-state and time-resolved fluorescence measurements were also performed.

The absorption spectra of **4**, **3** and **5** in water solutions are similar but not superimposible, showing a small red shift (from 333 to 335 nm) and a lowering of the molar absorptivity (3900, 3700 and 3600 $M^{-1}cm^{-1}$, respectively) in that order.

Figure 3 shows the fluorescence emission spectra of the two cyclodextrins **3** and **4**, measured under the same conditions, and that of the linear analogue **5**. Table 1 lists the corresponding excited state lifetimes and pre-exponential terms.

The fluorescence spectra of **3**, **4** and **5** in water solutions (Figure 3, normalized to the same relative intensity) show a trend very similar to that observed for the absorption spectra, but quantitatively more pronounced. A red shift in the

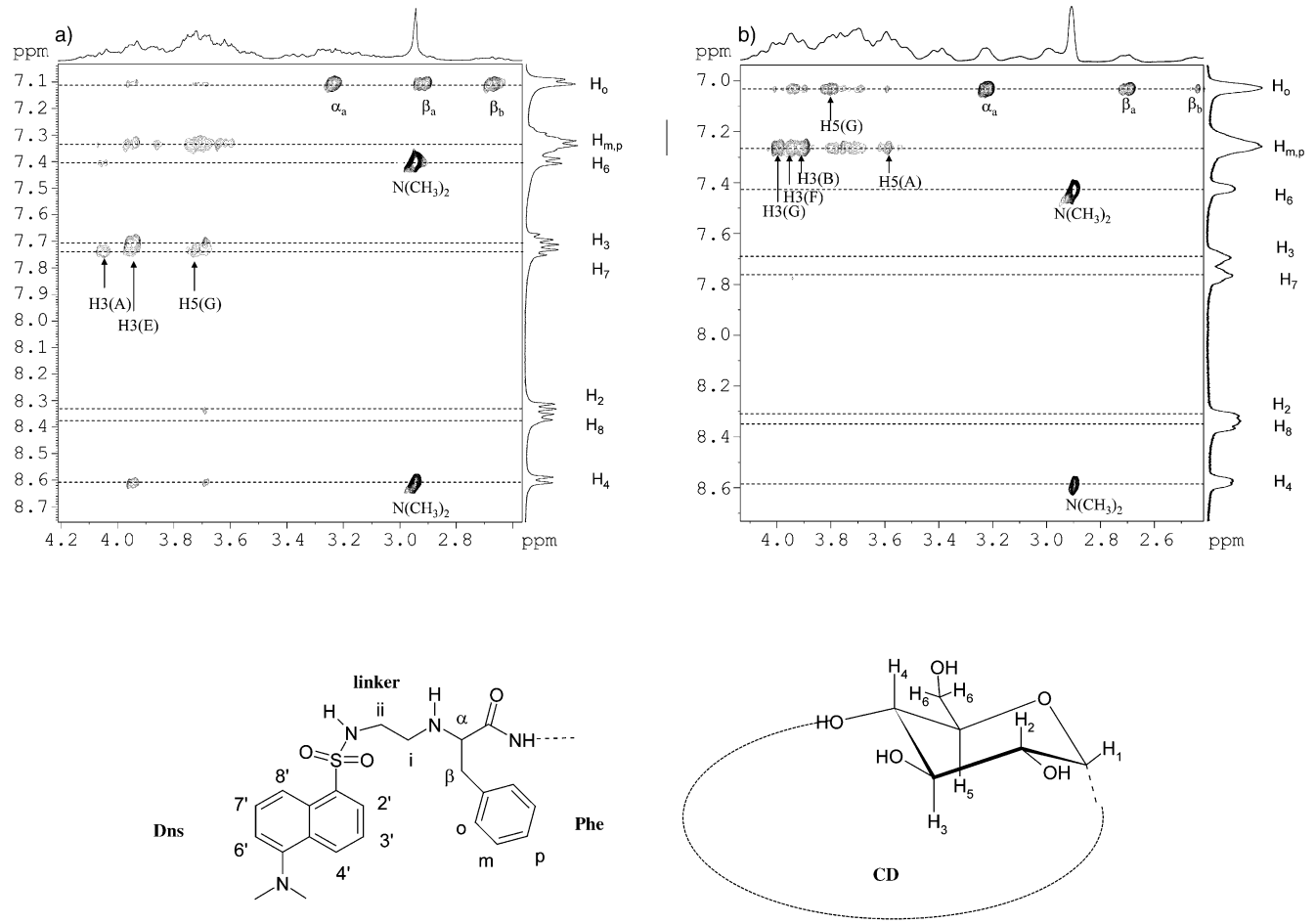


Figure 2. Portions of the ROESY 400 MHz spectra (D_2O, pH 7.0) of: a) **3**, b) **4**, showing cross-peaks between aromatic and aliphatic protons. A–G refer to different glucose rings.

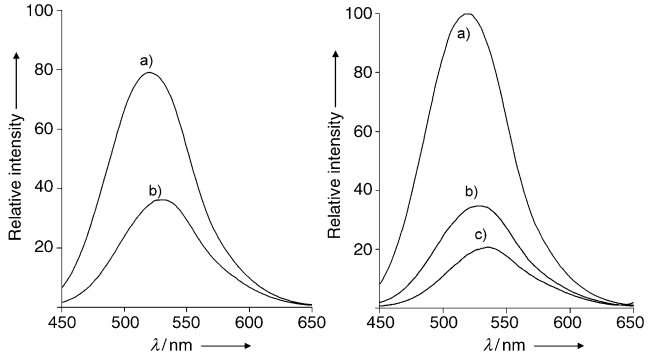


Figure 3. Fluorescence emission spectra. Left: a) **3** (6×10^{-5} M), b) **3** (6×10^{-5} M) after addition of a 50-fold excess of adamantane-1-carboxylic acid (ACA). Right: a) **4** (6×10^{-5} M), b) **4** (6×10^{-5} M) after addition of a 50-fold excess of ACA, c) **5** (6×10^{-5} M). Solvent: borate buffer 0.1 M, pH 7.3. λ_{ex} = 345 nm for **3** and **4**, 335 for **5**. Intensities are expressed as the percentage of the fluorescence of cyclodextrin **4** (corrected as described in the Experimental Section).

Table 1. Excited state lifetimes (τ) and pre-exponential terms (α) observed for samples of **3**, **4** and **5** solutions in aqueous borate buffer (0.1 M, pH 7.3), and of **5** in MeOH/H_2O 9:1 borate buffer (0.2 M, pH 7.9).

Sample	τ_A [ns]	α_A [%]	τ_B [ns]	α_B [%]
3	15	38	4	62
Cu-**3**	16	26	4	74
Cu-**3**/L-Pro[a]	16	30	4	70
Cu-**3**/D-Pro[a]	16	31	4	69
4	16	69	4	31
Cu-**4**	16	59	4	41
Cu-**4**/L-Pro[a]	17	51	5	49
Cu-**4**/D-Pro[a]	18	53	5	47
5	-	-	4	100
5[b]	-	-	13.2	100

[a] Copper(II)/cyclodextrin/amino acid 1:1:1.[b] Measured in MeOH/H_2O (9:1) borate buffer (0.2 M, pH 7.9).

fluorescence maximum is again observed on going from **4** to **3** and on to **5**, together with a decrease in the relative fluorescence intensity in the same order. In addition, while the excited state decay of **5** was strictly mono-exponential (τ = 4 ns), the decay of **3** and **4** can be conveniently fitted only if two different lifetimes are taken into account (τ_1 = 4 ns; τ_2 = 15 ns).

The charge-transfer excited state responsible for the dansyl fluorescence is very sensitive to the polarity of its environment; in particular, its maximum experiences a shift towards higher energy in apolar media, as shown by fluorescent measurements on other dansyl derivatives.[35] At the same time, increases in the fluorescence quantum yield and

in the excited state lifetime are observed, according to the shielding of the cyclodextrin cavity[19] and in agreement with the energy gap rule.[36]

From the presence of two different lifetimes, the overall fluorescence bands observed for **3** and **4** can be interpreted as a linear combination of two different bands: one with higher energy and longer lifetime, originating from dansyl units lying in a relatively apolar environment, and the other at lower energy with shorter lifetime, originating from units lying in a more polar environment.

The observed behaviour is thus consistent with a two-state model for the dansylated cyclodextrins, as previously described, with equilibrium between one conformation (out) in which the dansyl group is outside the cavity (more polar environment) and has a short fluorescence lifetime (τ_1), and one (in) with self-inclusion of the dansyl moiety (τ_2, more polar environment). The pre-exponential terms (α_1 and α_2), corresponding to the molar fractions of these two conformations, were found to be different for the two cyclodextrins: **3** showed only 38% of the "in" conformation, while **4** showed 69%. The difference in the normalized fluorescence intensities between the two cyclodextrins is thus due solely to the different extent of self-inclusion of the dansyl group, which is larger for **4**. This conclusion finds further support in analysis of the excited state lifetime of **5**, which increases from 4 to 16 ns in the presence of an excess of β-cyclodextrin, as a result of its inclusion in the cavity of the host.

Moreover, the addition of a 50:1 excess of 1-adamantanecarboxylic acid (ACA), which is known to form inclusion complexes with β-cyclodextrin, induced decreases in the fluorescence intensity for both **3** and **4** (Figure 3); after the addition, both cyclodextrins showed the same fluorescence maxima, suggesting that the inclusion of the guest had shifted the equilibrium towards the "out" for both **3** and **4**.

Similar results had previously been described by Ueno and co-workers for diastereomeric mono-functionalized β-cyclodextrins containing dansyl-D- or -L-leucine:[22] the latter was found to have a larger fraction ($\alpha = 0.77$) of the isomer showing a long excited state lifetime than the former ($\alpha = 0.67$), indicating that the chirality of the leucine residue could bias the conformational—and hence the molecular recognition and sensing—properties of these molecules. In the present case the difference in the distributions of the two conformations is much larger, suggesting that the self-inclusion of the dansyl moiety is more affected by the configuration of the phenylalanine linker.

The fluorescence properties of **5** in methanol/water (9:1) solutions are very similar to those typical of dansyl derivatives in methanol, and, as expected, its excited state decay was again strictly mono-exponential ($\tau = 13$ ns).

Copper(II) binding: Both cyclodextrins were shown to be quenched by copper(II) (Figure 4a); the quenching effects upon addition of Cu^{2+} in a 1:1 ratio were found to be in the same range for the two cyclodextrins.

It is important to note that in both cases a residual fluorescence band can still be observed even upon addition of one equivalent of metal ion. These bands have the same shapes and the same lifetimes (with the same distribution)

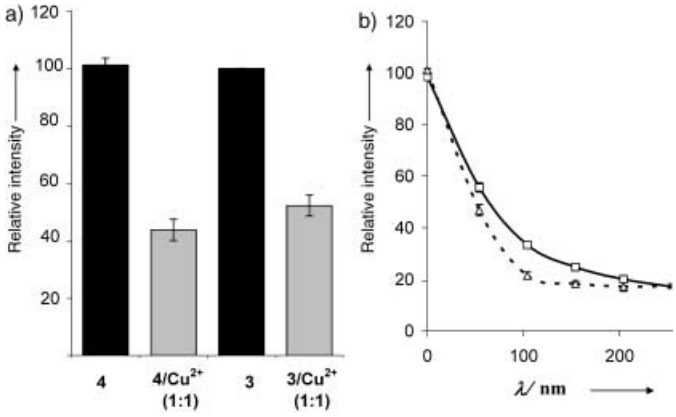


Figure 4. a) Fluorescence intensity decrease of **3** and **4** upon addition of $CuSO_4$ in 1:1 ratio, b) titration of **3** (□) and **4** (△) with $Cu(EDTA)^{2-}$ complex at pH 7.0. Concentration: 6×10^{-5} M, $\lambda_{ex} = 345$ nm, $\lambda_{em} = 516$ nm. Vertical bars indicate standard deviations.

as for the free **3** and **4**, indicating that they are due to uncomplexed ligand (Table 1). Since direct titration with excess of Cu^{2+} ion was unsuccessful, due to precipitation, competition experiments were performed with $Cu(EDTA)^{2-}$ as titrant (Figure 4b).

From these data the conditional equilibrium constants (K) for the equilibrium (1) at pH 7.0 could be calculated (K(**3**) = 0.020, s.d. = 0.002 and K(**4**) = 0.013, s.d. = 0.001). If it is assumed that no tertiary complexes are formed in these experiments, the stability of Cu-**3** can be gauged higher than that of Cu-**4**. This effect could be due to a lower degree of flexibility in **4** due to the inclusion of the dansyl group within the cavity.

$$Cu(EDTA)^{2-} + \mathbf{3}\ (\text{or } \mathbf{4}) \rightleftharpoons \text{Cu-}\mathbf{3}\ (\text{or Cu-}\mathbf{4}) + EDTA + n\,H^{+} \quad (1)$$

Enantioselective recognition of amino acids: The addition of amino acids to the copper(II) complexes of **3** or **4** in a 1:1 ratio caused increases in the fluorescence. These were dependent on the type of amino acid used and, for some amino acids, on the absolute configuration.[29]

The enantioselectivity displayed by the copper(II) complexes of cyclodextrins **3** and **4** was evaluated in terms of reproducibility, in order to ascertain whether and under which conditions the enantioselective effect might be useful for analytical applications. Three samples were analysed for each amino acid, to assess the significance of enantioselectivity observed at three different amino acid/copper(II) complex molar ratios (1:1, 2:1 and 10:1). The results obtained were compared by use of Student's t-test to verify the significance of the differences observed in fluorescence intensity (Table 2 and Table 3).

The best enantioselectivity was observed for proline with both cyclodextrins, and cyclodextrin **4** showed better enantiomeric recognition than **3**.

With **3**, good enantioselectivity and high significance were found only in the case of Pro for all the molar ratios utilized; Val and Ser gave significant differences at low amino

Table 2. Enantioselectivity factors ($\alpha = \Delta F_D/\Delta F_L$) of Cu-**3** (60 μM) towards amino acids (0.1 M borate buffer, pH 7.3; $\lambda_{ex} = 345$ nm, $\lambda_{em} = 516$ nm).

a.a.	[a.a.]:[Cu-**3**] = 1		[a.a.]:[Cu-**3**] = 2		[a.a.]:[Cu-**3**] = 10	
	α.	Signific. [%]	α.	Signific. [%]	α.	Signific. [%]
Pro	0.77	99	0.74	99	0.87	99
Ala	0.98	<20	0.85	99	0.94	80
Val	1.31	99	1.20	99	1.04	95
Leu	1.46	60	0.97	<20	1.39	99
His	1.05	60	1.00	<20	0.99	20
Asp	1.11	80	1.02	20	0.94	50
Lys	1.39	80	1.13	99	1.03	20
Ser	0.57	99	0.91	90	1.02	95
Phe	1.01	<20	1.02	<20	1.00	<20
Phgly	0.78	80	0.96	20	0.92	80
Trp	1.19	60	1.07	90	0.99	<20
Tyr	1.49	60	0.92	40	1.06	20

Table 3. Enantioselectivity factors ($\alpha = \Delta F_D/\Delta F_L$) of Cu-**4** and Cu-**5** towards amino acids.

a.a.	[a.a.]/[Cu-**4**(or **5**)] = 1		[a.a.]/[Cu-**4**(or **5**)] = 2		[a.a.]/[Cu-**4**(or **5**)] = 10	
	α	Signific. [%]	α	Signific. [%]	α	Signific. [%]
Cu-**4**[a]						
Pro	3.93	99	2.90	99	1.55	99
Ala	1.04	50	1.01	<20	0.99	40
Val	0.34	99	0.50	99	0.65	99
Leu	0.31	99	0.41	99	0.78	99
His	0.94	95	1.00	50	0.99	20
Asp	0.89	95	0.92	95	0.94	90
Lys	0.79	95	0.74	99	0.98	99
Ser	0.68	99	0.77	99	0.87	99
Phe	0.19	99	0.58	99	0.89	99
Phgly	0.43	99	0.70	95	0.87	99
Trp	0.62	95	0.77	99	0.98	90
Tyr	0.44	99	0.71	99	0.91	95
Cu-**5**[b]						
Pro	1.20	95	1.14	99	0.97	95
Phe	1.00	<20	0.95	50	1.00	40

[a] 60 μM in 0.1 M aqueous borate buffer, pH 7.3; $\lambda_{ex} = 345$ nm, $\lambda_{em} = 516$ nm. [b] 60 μM in MeOH/H_2O 9:1, 0.02 M borate buffer, pH 8.0; $\lambda_{ex} = 335$ nm, $\lambda_{em} = 535$ nm.

acid excess, while Leu gave a significant difference only at 10:1 excess. When cyclodextrin **4** was used instead, the copper complex gave good enantioselectivities for Pro, Val, Leu, Ser, Phe, and PhGly at all ratios, while Lys and Tyr enantiomers were discriminated only at low molar excess.

It is worth noting that alanine, with a small aliphatic side chain, produced negligible differences between the two enantiomers in fluorescence response, while the larger ring of proline and the aromatic moiety of phenylalanine induced enantiomeric differentiation. Large differences were also found in the case of tryptophan, but the large standard deviations obtained for this amino acid did not allow enantioselectivity to be assessed.

The linear ligand **5** was tested with Pro and Phe enantiomers, and the results showed remarkably lower enantioselectivity, thus indicating that the cyclodextrin cavity in **4** plays a role in determining the discriminating ability of the ligand (Table 3).

The good enantioselectivity obtained with proline for **4** allowed us to perform quantitative measurements of the enantiomeric purities of samples of intermediate composition by fast fluorescence measurements, with an accuracy within 5 %.[29]

The Cu-**3** and Cu-**4** complexes might therefore be usable as model systems for the development of enantioselective fluorescence chemosensors for unmodified amino acids for use in analytical applications. It was thus of great interest to understand the origin of the enantioselectivity and the sensing mechanism involved.

Mechanism of chiral discrimination: The observed enantioselectivity in the "switching on" process of the fluorescence of the two complexes can only be explained in terms of the formation of ternary cyclodextrin/copper(II)/amino acid complexes. The formation of these species was confirmed by ESI-MS measurements (see Supporting Information) performed under pH conditions comparable to those used for fluorescence experiments (pH 7.0). In the presence of **4**, copper(II) ions, and proline enantiomers, we detected signals for the ligand alone (as potassium adduct $[LK]^+$), for the copper(II) complex ($[CuLH_{-2}K_2]^{2+}$ and $[CuLH_{-2}K]^+$), and of the ternary complex ($[CuLH_{-1}(Pro)K]^+$ and $[CuLH_{-1}(Pro)K_2]^{2+}$). Therefore, both the amide and sulfonamide are deprotonated at this pH in the binary complex, while the ternary complex is mono-deprotonated, suggesting that the bidentate amino acid has displaced the sulfonamide group from the copper ion. The binary copper(II)/amino acid complex $[Cu(Pro)_2K]^+$ was also detected. Although the concentrations of the species were higher in these experiments than in the fluorescence measurements, due to the lower sensitivity of this technique, the formation of ternary complexes can be clearly demonstrated by this experiments.

Further evidence of the formation of ternary complexes and of their structures was provided by circular dichroism spectra (Figure 5).

The formation of the copper complex is accompanied by an increase in the signal intensity of the band at 350 nm, which indicated a more rigid structure, with the dansyl group equatorially oriented. A second band appeared at 280 nm, and was attributed to the deprotonated sulfonamide group, on the basis of circular dichroism spectra of the cyclodextrin **2** copper complex. This band appears at the same pH at which the deprotonation of the sulfonamide takes place (results not shown). Upon addition of one molar equivalent of D- or L-Pro, or of D- or L-Phe, the CD spectra showed a positive band at 350 nm, more intense than that of the free ligand and, in the case of L-Pro and D-Phe, of the copper(II) binary complex. At the same time, the band at 280 nm disappeared, indicating decomplexation of the sulfonamide group. The CD spectra therefore strongly support the hypothesis of formation of ternary complexes.

In order to understand the mechanism involved in the enantioselective sensing, we investigated the excited state lifetimes of the dansyl chromophore in the Cu/**3**/amino acid (1:1:1) and Cu/**4**/amino acid (1:1:1) mixtures (Table 1). In each case we found that the same two lifetimes with the same pre-exponential terms as the free ligand were also

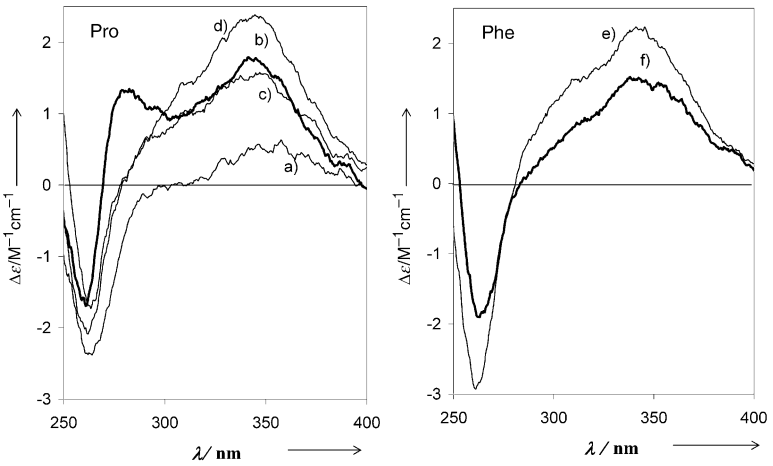


Figure 5. Circular dichroism spectra of: a) **4**, b) Cu/**4** (1:1), c) Cu/**4**/D-Pro (1:1:1), d) Cu/**4**/L-Pro (1:1:1), e) Cu/**4**/D-Phe (1:1:1), f) Cu/**4**/L-Phe (1:1:1) in aqueous borate buffer, pH 7.0 at concentrations of 6×10^{-5} M.

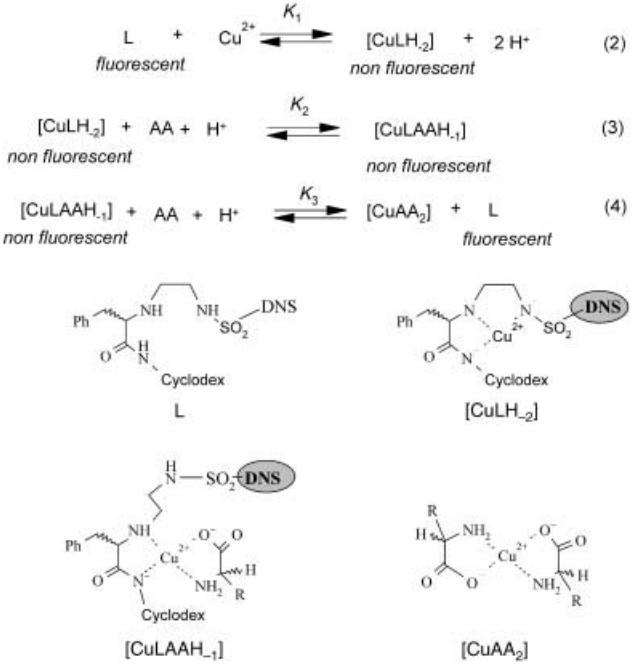
present after the addition of the amino acid. From these results, we believe that the dansyl fluorescence is almost completely quenched in the ternary complex. It seems very unlikely that each ternary complex could have the same photophysical properties and the same conformer distribution as the parent free ligand. In addition, the copper ion in the ternary complex lies very close to the chromophore, and for this reason, it can still reasonably produce efficient energy- or electron-transfer processes. In a previous study we demonstrated that simple dansylated polyamine ligands could bind copper(II) over a wide pH range.[35] The fluorescence of the dansyl group was quenched when copper(II) complexes were formed, as well as in species in which the dansyl group was not coordinated to the metal ion. Therefore, a quenching process of the dansyl group involving the copper(II) ion is still effective in the ternary complex, though the dansyl group is displaced by the metal ion, as indicated by its neutral charge (observed in the ESI spectrum) and by circular dichroism studies.

The observed sensing process can therefore be interpreted in terms of a simple model as shown in Scheme 2, Equilibria (2)–(4).

Equilibrium (2) is copper(II) complexation, and is dependent on the conformation of the ligand, being more effective for the cyclodextrin **4**, as shown by the quenching experiments reported in Figure 3. The "switch on" process is then composed of two equilibria: Equilibrium (3) the formation of a non-fluorescent ternary complex, and Equilibrium (4) the displacement of copper(II) by the amino acid to form a 1:2 complex with displacement of the free fluorescent ligand. The enantioselectivity in the fluorescence "switching on" is therefore based on competition between the non-fluorescent ternary complex and the binary copper(II):amino acid complex, since $K_2(\text{L}) \neq K_2(\text{D})$ and $K_3(\text{L}) \neq K_3(\text{D})$.

From this scheme, enantioselectivity could also be observed in the reverse process reported in Equilibrium (4), by titration of the cyclodextrin **4** with the 2:1 copper(II) complex of the amino acid. Figure 6 reports the quenching observed during the titration experiment, which allows the difference in the stability constants of the ternary diastereomeric complexes formed to be evaluated.

According to this model, the high ratio of the association constants for the two enantiomers ($K_{SV}(\text{L})/K_{SV}(\text{D}) = 2.36$) corresponds to the ratio between the overall stability constants of the diastereomeric ternary complexes, since the



Scheme 2. Equilibria involved in the sensing process and proposed copper(II) coordination in the various species.

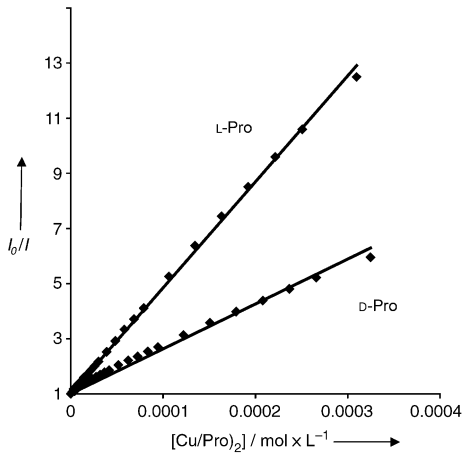


Figure 6. Stern–Volmer plot obtained by quenching the fluorescence of **4** (6×10^{-5} M) with Cu(L-Pro)$_2$ (upper line) or Cu(D-Pro)$_2$ (lower line) in 0.1 M borate buffer, pH 7.3.

binary species have the same stability for the two enantiomers.

The higher enantioselectivity observed in the case of **4** can be explained by taking the conformational properties of the ligand into account: the higher degree of self-inclusion of the dansyl group within the cyclodextrin cavity did not affect the copper(II) binding ability, suggesting that in the copper(II) complex the dansyl group retained the same conformation. A more rigid ternary complex could therefore be formed, and the different orientation of the amino acid side chain with respect to the cavity could give rise to different interactions. The same effect could not be obtained with the cyclodextrin **3**, which has the dansyl group outside the cavity, or by the linear ligand **5**, which has the same configuration as **4** but lacks the pre-organization induced by the cyclodextrin cavity.

Conclusion

Rationally designed enantioselective fluorescent sensors are of great importance in the development of new analytical tools for assessment of optical purity. Herein we have demonstrated that the combination of a fluorophore, a metal binding site and a lipophilic cavity gives rise to high enantioselectivity for some amino acids. The mechanism of sensing can be attributed to competition between the ternary copper(II)/ligand/amino acid complex and the binary copper(II)/amino acid complex.

Our results indicated that a highly pre-organized structure can be achieved by allowing self-inclusion of the fluorophore in the cyclodextrin cavity, thus giving rise to more rigid structures. The stereochemistry of the side arm used was found to be important, in the present case, for determination of the conformational properties of ligands **3** and **4**, since they affect the ability to complex copper(II) and to discriminate between enantiomers of amino acids. Other selectors designed with the same structural features are being synthesized in order to obtain a pool of selectors for parallel analysis.

The proposed method can be used for analytical purposes, at least when fast screening procedures are required. Unlike many other proposed methods for parallel analysis of enantiomers, the present one is based not on kinetic enantiodiscrimination, but on enantioselectivity in complex formation. Therefore, only a simple mixing procedure, which can easily be automated, is required. Parallel read-out devices can be used for this purpose. Future development could be envisaged for chiral analysis of unmodified natural or synthetic amino acids, providing new and efficient tools for screening of synthetic processes or of enantioselective catalysts both in traditional organic synthesis and in combinatorial chemistry.

Experimental Section

General: Starting materials were obtained from commercial suppliers and were used without further purification. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. TLC chromatography was performed on precoated silica gel SIL G/UV_{254} aluminium support (0.20 mm layer; Macherey Nagel). HPLC chromatography was performed on a Waters HPLC Chromatograph, equipped with a model 4000 pump and a model 2487 UV detector, set at 330 nm, with a C18 Spherisorb column (25×200 mm). IR spectra were recorded on a Nicolet 5PC FT-IR spectrometer, and ^{1}H NMR and ^{13}C NMR were recorded on Bruker AC 300 or AMX 400 spectrometers. The optical purities of **3**, **4** and **5** were checked by GC/MS analysis on a Hewlett–Packard HP 6890 chromatograph fitted with a Hewlett Packard HP 5973 detector and a Chirasil-Val column. ESI-MS experiments were performed on a Micromass ZMD mass spectrometer (Micromass, Manchester, UK), fitted with an electrospray source and a single quadrupole mass analyser. Data were acquired by use of Masslynx 3.4 software (Micromass, Manchester, UK). UV spectra were recorded on a Perkin–Elmer Lambda 40 spectrophotometer. CD spectra were obtained on a Jasco 715A spectropolarimeter. Fluorescence spectra were recorded on a Perkin–Elmer Ls50B instrument in a 1×0.2 cm quartz cell thermostatted at 25 °C. The fluorescence lifetimes (uncertainty ±5%) were obtained with an Edinburgh single-photon counting apparatus (D_2-filled flash lamp). To allow comparison of emission intensities, corrections were performed for instrumental response, inner filter effects, and phototube sensitivity. Elemental analysis were performed with a Carlo Erba CHNS-O EA1108 elemental analyzer.

***N*$^{\alpha}$-[*N*^{2}-(*tert*-Butoxycarbonyl)aminoethyl]-D(or L)-phenylalanine (D-6 or L-6)**: These compounds were synthesized as described previously.[37]

***N*$^{\alpha}$-[*N*^{2}-(5-Dimethylamino-1-naphthalenesulfonyl)aminoethyl]-D (or L)-phenylalanine (D-8 and L-8)**: Anisole (0.49 mL, 4.52 mmol) and D-**6** (or L-**6**) (0.3 g, 0.75 mmol) were dissolved in CH_2Cl_2 (30 mL) and cooled to 0 °C. $AlCl_3$ (0.6 g, 4.52 mmol) in CH_3NO_2 (15 mL) was then added with stirring. The mixture was stirred at room temperature for 15 min, and the reaction was stopped by addition of few mL of water. The solution was extracted with CH_2Cl_2 to remove anisole, the pH of the aqueous phase was adjusted to 8, and the formed $Al(OH)_3$ precipitate was removed by filtration. The filtrate was evaporated to dryness to yield a solid residue, which was dissolved in a solution of Li_2CO_3 (0.17 g, 2.25 mmol) in water (10 mL, pH 9.5), prepared immediately before use. Dansyl chloride (0.20 g, 0.75 mmol) in CH_3CN (10 mL) was added dropwise at 0 °C (0.25 equiv per hour). Unreacted dansyl chloride was removed by extraction with diethyl ether. The product was precipitated after acidification (pH 8) of the aqueous layer. Yield 69%. R_f = 0.55 (BuOH/H_2O/acetic acid 4:1:1).

Compound D-8: M.p. 200–215 °C (decomp); $[\alpha]_D^{20} = -9.7$ (c = 1 in water, pH 12); compound L-**8**: m.p. 200–215 °C (decomp); $[\alpha]_D^{20} = +10.5$ (c = 1 in water, pH 12); ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 2.18–2.26 (m, 3H), 2.41–2.50 (m, 1H), 2.89 (s, 6H; $N(CH_3)_2$), 2.78–3.01 (m, 3H; N–CH, SO_2N-CH_2), 6.97–7.01 (m, 2H; Ph), 7.24–7.30 (m, 3H; Ph), 7.43 (d, ^3J(H,H) = 7.4 Hz, 1H; $H_{6'}$), 7.68 (t, ^3J(H,H) = 8.4 Hz, 1H; $H_{3'}$), 7.75 (t, ^3J(H,H) = 7.8 Hz, 1H; $H_{7'}$), 8.14 (d, ^3J(H,H) = 7.1 Hz, 1H; $H_{8'}$), 8.39 (d, ^3J(H,H) = 8.4 Hz, 1H; $H_{2'}$), 8.69 (d, ^3J(H,H) = 8.6 Hz, 1H; $H_{4'}$) ppm; ^{13}C NMR (75 MHz, D_2O, 25 °C): δ = 42.08, 47.92, 48.29, 51.13, 68.51, 118.83, 124.61, 127.44, 129.69, 130.70, 130.93, 131.31, 131.68, 132.29, 132.35, 133.18, 141.41, 142.11, 153.55, 184.31 ppm; IR (KBr): $\tilde{\nu}$ = 3308 cm^{-1} (N–H), 1574 cm^{-1} (C=O), 1323 cm^{-1} (S=O); MS (C.I.): m/z (%): 442 (12) $[M+H]^+$, 171 (57) [dimethylaminonaphthalene+H]$^+$, 122 (100) $[C_8H_{12}N]^+$; elemental analysis calcd (%) for $C_{23}H_{27}N_3O_4S$ (441.54): C 62.57, H 6.16, N 9.42; found: C 62.20, H 6.41, N 9.42.

6-Deoxy-6-*N*-(*N*$^{\alpha}$-[(5-dimethylamino-1-naphthalenesulfonyl)aminoethyl]-phenylalanylamino-β-cyclodextrin (3, 4): HBTU (0.024 g, 0.062 mmol) and mono-6-deoxy-6-amino-β-cyclodextrin hydrochloride (0.066 g, 0.056 mmol) were dissolved in dry DMF (4 mL) at 0 °C. Compound D-**8** (or L-**8**; 0.027 g, 0.062 mmol) and DIEA (0.031 mL, 0.180 mmol) were added in small portions every five minutes. The reaction was stopped after three hours by addition of few mL of water. Water and DMF were evaporated under vacuum at 40 °C. The obtained yellow solid was dissolved in water, and the insoluble residue, unreacted **8**, was removed by filtration. The product was precipitated in acetone and purified by HPLC chromatography; the mobile phase was water (eluent A) and CH_3CN/TFA (0.1%) (eluent B), with a linear gradient from 100% of A to 100% of B in 10 minutes with a flow rate of 20 mL min^{-1}; retention times of **3**

or **4** were 8–8.5 minutes. The products were thus isolated and characterized as trifluoroacetate salts.

Compound 3: Yield 22%. R_f = 0.56 (PrOH/H_2O/NH_3 5:3:1). M.p. 206°C (decomp); *ee* = 95%; ^{1}H NMR (300 MHz, D_2O, 25°C): δ = 2.78 (m, 1H), 2.96 (m, 1H), 3.04 (s, 6H; $N(CH_3)_2$), 3.23–3.42 (m, 6H), 3.55–3.88 (m, 26H), 3.93–4.16 (m, 14H), 4.93 (d, ^3J(H,H) = 3.4 Hz, 1H; H_1), 5.05 (d, ^3J(H,H) = 3.4 Hz, 1H; H_1), 5.10 (d, ^3J(H,H) = 3.4 Hz, 1H; H_1), 5.13 (d, ^3J(H,H) = 3.7 Hz, 1H; H_1), 5.19 (d, ^3J(H,H) = 3.4 Hz, 2H; H_1), 5.23 (d, ^3J(H,H) = 3.5 Hz, 1H; H_1), 7.15–7.25 (m, 2H; Ph), 7.40–7.50 (m, 3H; Ph), 7.57 (d, ^3J(H,H) = 7.6 Hz, 1H; $H_{6'}$), 7.84 (t, ^3J(H,H) = 8.6 Hz, 1H; $H_{3'}$), 7.87 (t, ^3J(H,H) = 8.7 Hz, 1H; $H_{7'}$), 8.40 (d, ^3J(H,H) = 7.5 Hz, 1H; $H_{2'}$), 8.44 (d, ^3J(H,H) = 8.7 Hz, 1H; $H_{8'}$), 8.65 (d, ^3J(H,H) = 8.6 Hz, 1H; $H_{4'}$) ppm; ^{13}C NMR (75 MHz, D_2O, 25°C): δ = 40.77, 43.56, 48.56, 49.10, 62.88, 63.23, 63.37, 63.53, 65.44, 74.49, 74.90, 74.98, 75.09, 75.56, 75.91, 76.20, 76.46, 76.63, 83.10, 83.53, 83.79, 84.29, 84.38, 85.97, 103.76, 104.76, 105.07, 105.31, 119.17, 122.35, 127.43, 130.82, 131.93, 132.27, 132.44, 133.11, 133.61, 137.08, 138.42, 154.53 ppm; IR (KBr): $\tilde{\nu}$ = 3650–3050 cm^{-1} (O–H, N–H), 1650 cm^{-1} (C=O), 1150 cm^{-1} (S=O); MS (ESI): *m*/*z* (%): 1558 (100) $[M+H]^+$, 1579 (20) $[M+Na]^+$, 1597 (26) $[M+K]^+$; elemental analysis calcd for $C_{69}H_{98}N_4O_{41}SF_6 \cdot 1H_2O$ (1804): C 45.95, H 5.59, N 3.11; found: C 45.87, H 5.28, N 3.23.

Compound 4: Yield 29%. R_f = 0.56 (PrOH/H_2O/NH_3 5:3:1); m.p. 200°C (decomp); *ee* = 94%; ^{1}H NMR (300 MHz, D_2O, 25°C): δ = 3.10–4.05 (m, 53H), 4.15 (m, 1H), 4.33 (m, 1H), 5.00–5.20 (m, 7H; H_1), 7.25–7.34 (m, 2H; Ph), 7.45–7.58 (m, 3H; Ph), 8.07–8.12 (m, 2H; $H_{3'}$, $H_{7'}$), 8.27 (d, ^3J(H,H) = 7.8 Hz, 1H; $H_{6'}$), 8.46 (d, ^3J(H,H) = 7.5 Hz, 1H; $H_{8'}$), 8.64 (d, ^3J(H,H) = 8.4 Hz, 1H; $H_{2'}$), 8.93 ppm (d, ^3J(H,H) = 8.7 Hz, 1H; $H_{4'}$); ^{13}C NMR (75 MHz, D_2O, 25°C): δ = 39.70, 42.25, 45.22, 50.17, 63.51, 63.66, 63.83, 64.45, 74.79, 74.99, 75.16, 75.21, 75.35, 75.53, 75.77, 75.90, 76.31, 76.43, 76.48, 76.60, 84.15, 84.51, 84.57, 85.06, 85.47, 88.15, 104.57, 105.00, 105.37, 105.46, 105.51, 105.63, 119.84 (q, ^1J(C,F) = 289.7 Hz; CF_3), 123.12, 129.17, 129.25, 129.86, 130.32, 131.69, 131.80, 132.06, 132.40, 132.47, 136.48, 141.97, 166.29 (q, ^3J(C,F) = 34.6 Hz; *C*OCF_3), 170.70 ppm; IR (KBr): $\tilde{\nu}$ = 3650–3050 cm^{-1} (O–H, N–H), 1683 cm^{-1} (C=O), 1150 cm^{-1} (S=O); MS (ESI): *m*/*z* (%): 1558 (100) $[M+H]^+$, 798 (65) $[M+H+K]^{2+}$, 779 (83) $[M+2H]^{2+}$; elemental analysis calcd for $C_{67}H_{97}N_4O_{39}SF_3 \cdot 15H_2O$ (1941): C 41.46, H 6.54, N 2.89; found: C 41.42, H 6.82, N 3.03.

***N*^{1}-Propyl-*N*$^\alpha$-[*N*^{2}-(5-dimethylamino-1-naphthalenesulfonyl)aminoethyl]-L-phenylalaninamide (5)**: HBTU (0.171 g, 0.451 mmol) and *n*-propylamine hydrochloride (0.058 g, 0.607 mmol) were dissolved in dry DMF. Compound L-**8** (0.132 g, 0.300 mmol) and DIEA (0.235 mL, 1.350 mmol) were added in small portions every five minutes over one hour. The reaction was stopped by addition of few mL of water. Water and DMF were evaporated under vacuum at 40°C. The yellow solid obtained was dissolved in water at pH 2 and extracted with chloroform; the organic layer was washed with basic water (pH 8), dried over $MgSO_4$ and evaporated to dryness. The product was purified by HPLC: the mobile phase was MeOH/H_2O 95:5 (v/v) with TFA (0.035%), flow rate = 18 mL min^{-1}: retention time of **5** was 4.5 min. The product was isolated as a trifluoroacetate salt, dissolved in water at pH 8 and extracted in chloroform; it was then treated with a HCl/methanol solution and precipitated with methanol/diethyl ether.

Compound 5: Yield 48%. R_f = 0.30 (ethyl acetate/hexane 1:1, TFA 0.1%); m.p. 168°C; *ee* = 91%; ^{1}H NMR (300 MHz, CD_3OD, 25°C): δ = 0.72 (t, ^3J(H,H) = 7.4 Hz, 3H; CH_3 propyl), 1.24–1.34 (m, ^3J(H,H) = 7.4 Hz, ^3J(H,H) = 7.2 Hz, 2H; CH_2 propyl), 2.94–3.28 (m, 8H; CH_2 propyl, CH_2Ph, CH_2N), 3.11 (s, 6H; $N(CH_3)_2$), 4.06 (dd, ^3J(H,H) = 9.8 Hz, ^3J(H,H) = 5.3 Hz, 1H; CH), 7.19–7.37 (m, 5H; Ph), 7.59 (d, ^3J(H,H) = 7.7 Hz, 1H; $H_{6'}$), 7.69–7.75 (m, 2H; $H_{3'}$, $H_{7'}$), 8.30 (dd, ^3J(H,H) = 7.3 Hz, ^4J(H,H) = 1.1 Hz, 1H; $H_{8'}$), 8.53 (d, ^3J(H,H) = 8.6 Hz, 1H; $H_{2'}$), 8.60 (d, ^3J(H,H) = 8.6 Hz, 1H; $H_{4'}$) ppm; ^{13}C NMR (75 MHz, CD_3OD, 25°C): δ = 11.88, 23.40, 37.99, 40.20, 42.68, 46.70, 47.71, 63.43, 118.31, 123.17, 125.83, 129.06, 129.63, 130.25, 130.41, 130.64, 130.82, 130.99, 131.19, 135.53, 136.68, 149.41, 169.03 ppm; IR (KBr): $\tilde{\nu}$ = 3266 cm^{-1} (N–H), 3066 cm^{-1} (C–H), 2964 cm^{-1} (C–H), 1683 cm^{-1} (C=O), 1566 cm^{-1} (N–H), 1337 cm^{-1} (S=O), 1147 cm^{-1} (S=O); MS (CI): *m*/*z* (%): 483 (100) $[M]^+$, 396 (67) $[M-(CONHPr)]^+$, 170 (35) [dimethylaminonaphthalene]$^+$; elemental analysis calcd for $C_{26}H_{34}N_4O_3S \cdot 2HCl \cdot {}^3/_4 H_2O$ (569.07): C 54.87, H 6.64, N 9.85; found: C 54.59, H 6.27, N 9.53.

Fluorescence measurements: Concentrated stock solutions of **3** and **4** (1×10^{-3} M in water) and **5** (1×10^{-3} M in methanol) were prepared; solutions of **3** and **4** were diluted to final concentrations of 6×10^{-5} M at pH 7.3 in 0.1 M borate buffer, the solution of **5** was diluted to a final concentration of 6×10^{-5} M at pH 8.0 methanol/water (9:1 v/v, 0.02 M borate buffer). In the titration experiments with copper(II), 0.5 mL aliquots of these solutions were titrated in the cell by addition of 6×10^{-3} M $CuSO_4$ in water with a 10 μL syringe. Three measurements were performed. The same procedure was followed in the titrations of **3** and **4** with $Cu(EDTA)^{2-}$ (6×10^{-2} M in water), adamantanecarboxylic acid (ACA, 6×10^{-2} M in methanol) and in the titration of **4** with $Cu(Pro)_2$ (6×10^{-2} M in water). Fluorescence intensities were corrected by a literature procedure;[38] a futher correction of the fluorescence intensity of all the samples was made according to $I_n = I/I_{st}$, where I is the observed fluorescence intensity and I_{st} is the intensity of a reference solution of **4**, both measured at the same excitation and emission wavelength.

Fluorescence intensities from the titration with $Cu(EDTA)^{2-}$ were used according to the following model. By considering that [EDTA] = [Cu-**3** (or Cu-**4**)] in equilibrium (1) (i.e., by neglecting the dissociation of the Cu(EDTA) complex, due to its high stability) and by keeping the pH buffered, Equation (5) could be obtained, where I is the corrected fluorescence intensity, I_0 is the fluorescence of each cyclodextrin alone, C_{CD} and $C_{Cu(EDTA)}$ are the analytical concentrations of the cyclodextrin and of $Cu(EDTA)^{2-}$ respectively, and $A = KC_{Cu(EDTA)} - KC_{CD} + 2C_{CD}$.

$$I/I_0 = \frac{-A + [A^2 + 4(KC_{CD} - C_{CD})C_{CD}]^{1/2}}{2(KC_{CD} - C_{CD})} \quad (5)$$

Nonlinear regressions of I/I_0 values were performed with the aid of the Sigmaplot program (18 data points for each cyclodextrin, from three independent measurements).

Titrations of Cu-**3** and Cu-**4** with amino acids were carried out with 6×10^{-5} M solutions of the complexes in 0.1 M borate buffer at pH 7. To 0.5 mL of these solutions were added aliquots of D- or L-amino acids (6×10^{-2} M in water); each titration was repeated three times: mean values and standard deviations were calculated. During each titration, the pH was constant, as verified by a combined glass electrode.

Samples analyzed by time-resolved fluorescence were prepared in the same way; amino acids were added in tenfold excess to the solutions of Cu-**3** and Cu-**4**.

UV and CD measurements: Samples for UV and CD measurements were prepared as described for fluorescence measurements.

2D NMR spectroscopy: 2D spectra were performed at 400 MHz on a Bruker AMX 400 spectrometer. Compounds **3** and **4** were dissolved in D_2O at concentrations of 10 mM and the pH was adjusted to 7 with NaOD. The corresponding ^{1}H chemical shifts observed in the 2D experiments were different from those reported above in the characterization part, due to different degrees of protonation; the signal assignments are based on TOCSY and ROESY spectra. The mlevtp pulse program was used for the TOCSY experiments, with different mixing times (30, 60 and 90 ms). The roesysh pulse program was used for the ROESY experiments, with 250 ms mixing time.

ESI-MS measurements: Solutions for MS experiments were prepared by diluting standard solutions of **3** or **4**, $CuSO_4$, L- and D-proline to final concentrations of 5×10^{-4} M in water: solutions were basified to pH 7 by addition of 0.1 M KOH.

ESI-MS spectra were recorded in positive ion mode by perfusion of the solutions directly into the mass spectrometer at a flow rate = 10 μL min^{-1} (conditions: capillary voltage 3000 V, cone voltage 50 V, desolvation flow (N_2) = 500 L h^{-1}, nebulizer flow (N_2) = 100 L h^{-1}, desolvation temperature = 80°C, source block temperature = 150°C, scan range *m*/*z* 200–2000 Da, scan time 4 s).

GC-MS measurements: The enantiomeric excesses of the ligands **3**, **4** and **5** were checked by GC/MS analysis of the corresponding 4-*N*-trifluoroacetyl-3-benzylpiperazine-2-ones.The ligands (1–2 mg) were hydrolysed at 100°C with HCl (6 N, 2 mL) for 6 h to the corresponding *N*-2-aminoethylphenylalanine, then suspended in CH_2Cl_2 (2 mL) and treated with trifluoroacetic anhydride (0.3 mL) for 1 hour at 60°C. After removal of excess reagent by evaporation under vacuum, the samples were dissolved in CH_2Cl_2 and injected into the GC (1 μL). Analysis were performed

with a chiral capillary column: Chirasil-Val, 10 m, I.D. = 0.25 mm, film thickness: 0.12 μm. carrier: He. Flow: 1.1 mL min^{-1}. injector temperature: 230 °C. detector temperature: 230 °C.; isotherm 180 °C, detection: MS, SIM mode (*m*/*z* 91, 286, 167).

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