Modified Cyclodextrins as Enantioselective Hosts for Amino Acids

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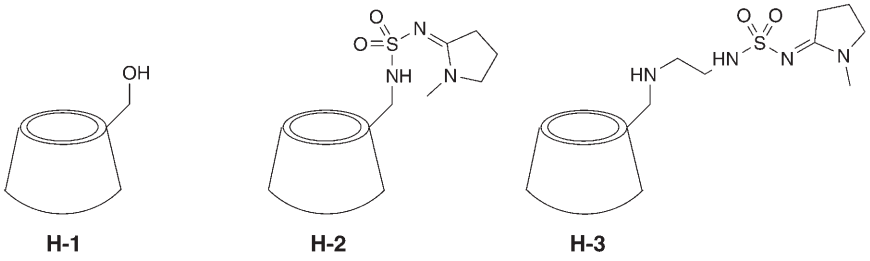
Two modified β-cyclodextrins, **H-2** and **H-3**, having a flexible appended moiety were studied for the chiral discrimination of the enantiomers of various amino acids by means of fluorescence as signaling option. These hosts quenched the fluorescence intensities of amino acids upon binding. The D-enantiomers were better recognized by these hosts. The association constants (K_s) and enantioselectivity factors (α) of the host·guest complexes were calculated.

Introduction. – Chiral discrimination is a field of great interest in the present day biotechnology, especially in drug design and synthesis [1]. Amongst various approaches, solution-based systems capable of chiral recognition are of immense pharmaceutical potential [2]. Enantioselective hosts for chiral discrimination by fluorescence as signaling option have various advantages [3]. Amongst them, fluorescent hosts based on molecular recognition are of great interest due to high sensitivity and selectivity and also due to their ability to detect various organic molecules. Enantioselective fluorescent discrimination with chiral hosts offers a variety of advantages over traditional chromatographic and NMR spectroscopic techniques such as high sensitivity, low cost of instrumentation, waste reduction, time efficiency, and the possibility of real-time analysis.

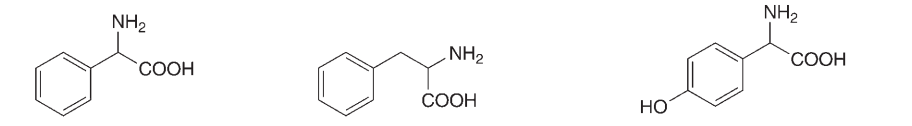
Since the cyclodextrin (CD) cavity, apart from being hydrophobic, is also chiral in nature, various derivatized CDs can be further explored for chiral recognition of organic molecules. The advantages of using derivatized CDs are: *a*) change in fluorescence intensity upon inclusion in the CD cavity [4]; *b*) recognition of apolar groups in H_2O by the hydrophobic cavity of CD; *c*) enantioselective recognition by formation of diastereoisomeric complexes with enantiomers of different molecules due to the chiral nature of CD; *d*) above all, CD derivatives are soluble in H_2O. Recently, *Corradini* and co-workers have reported the ability of chiral discrimination of various amino acid derivatives by metal-bound dansyl-modified cyclodextrins [5].

Since enantiomer recognition is based on the configuration of the chiral substrate, we undertook a study with derivatized chiral CDs for the enantiomer recognition of amino acids by means of fluorescence as signaling option. In our previous report [6], the two CD derivatives mono{6-deoxy-6-{{[(1-methylpyrrolidin-2-ylidene)amino]sulfonyl}amino}}-β-cyclodextrin (**H-2**), mono{6-deoxy-6-{{2-{{[(1-methylpyrrolidin-2-ylidene)amino]sulfonyl}amino}ethyl}amino}}-β-cyclodextrin (**H-3**) were shown to be

efficient enantiomer-discriminating hosts for (*R*,*S*)-zolmitriptan. Now, we wish to report their enantiomer-discriminating property for various amino acids. The CDs **H-2** and **H-3** have a flexible appended moiety which sticks down into the hydrophobic cavity of CD and is displaced by the guest from the hydrophobic cavity to the hydrophilic environment of the CD.



H-1 **H-2** **H-3**



D-**1** D-2-Phenylglycine
L-**1** L-2-Phenylglycine

D-**2** D-Phenylalanine
L-**2** L-Phenylalanine

D-**3** D-2-(4-Hydroxyphenyl)glycine
L-**3** L-2-(4-Hydroxyphenyl)glycine

D-**4** 3,4-Dihydroxy-D-phenylalanine
L-**4** 3,4-Dihydroxy-L-phenylalanine

D-**5** D-Tryptophan
L-**5** L-Tryptophan

Results and Discussions. – A systematic study of the native β-cyclodextrin (**H-1**) and the modified cyclodextrins **H-2** and **H-3** was undertaken for the enantiomer recognition of the amino acids D/L-2-phenylglycine (D/L-**1**), D/L-phenylalanine (D/L-**2**), D/L-2-(4-hydroxyphenyl)glycine (D/L-**3**), 3,4-dihydroxy-D/L-phenylalanine (D/L-**4**), and D/L-tryptophan (D/L-**5**).

The fluorescence spectral data of the guests in the presence of different compositions of the hosts in 0.1M tetraborate buffer were evaluated. All the amino acids experienced quenching of the fluorescence intensity upon addition of various concentrations of the hosts **H-1**, **H-2**, and **H-3**. Enantioselectivity in fluorescence quenching was observed with all the amino acids studied. The quenching of the fluorescence intensity of the D-enantiomer was higher than that of the L-enantiomer in the presence of **H-2** and **H-3**. The fluorescence spectra of L-**4** (*Fig. 1*,*a*), D-**4** (*Fig. 1*,*b*), L-**5** (*Fig. 1*,*c*), and D-**5** (*Fig. 1*,*d*) in 0.1M tetraborate buffer (pH 7.2) in the absence and presence of **H-2** showed that the fluorescence intensity of the guests decreases with increasing host concentration. A similar pattern of quenching was observed in the case of all other amino acids with the hosts. These changes of fluorescence intensity induced

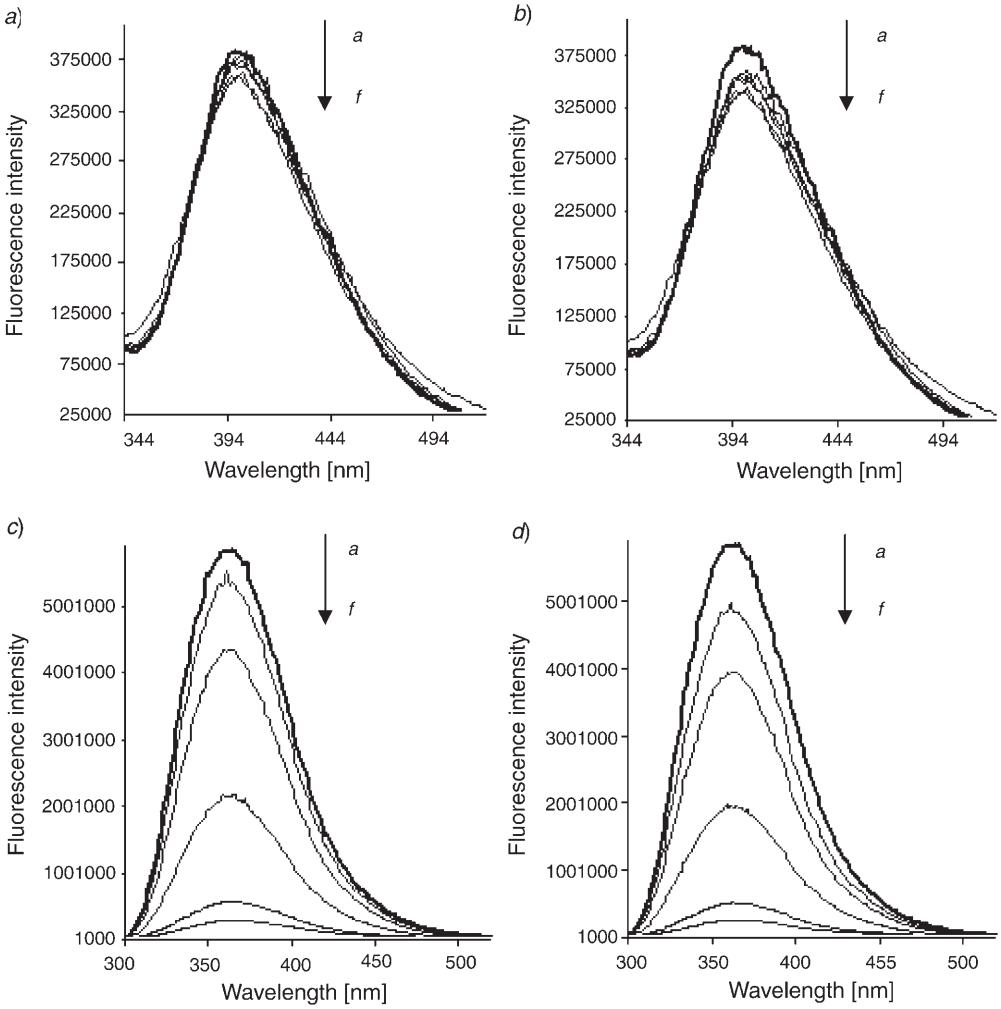


Fig. 1. *Fluorescence spectra* of a) L-**4**, b) D-**4**, c) L-**5**, *and* d) D-**5** *in the absence and presence of various concentrations of the host* **H-2**. The concentration of **H-2** ranged from 0 to 0.1, 0.2, 0.5, 1.0, and 1.5mM (from *a* to *f*), and the concentrations of the guests were $1 \cdot 10^{-4}$ M.

by all the hosts indicated that the hosts and amino acids formed host · guest inclusion complexes.

To evaluate the extent of enantiomer recognition of these hosts, the enantioselectivity factor $\alpha = \Delta F_D/\Delta F_L$ [7] (*Table 1*) of all the guests **1**–**5** with the hosts **H-2** and **H-3** were calculated; ΔF_D is $F_0 - F_D$, and ΔF_L is $F_0 - F_L$, with F_0 = fluorescence intensity of the guest alone, F_D = fluorescence intensity of the host · D-isomer complex, and F_L = fluorescence intensity of the host · L-isomer complex. For all the guests studied, the value of α is in the range of 1.95 to 1.06, except for guest **1**, which exhibits the highest

value with **H-2** (3.79) and lowest value with **H-3** (0.45). Host **H-1** did not discriminate the enantiomers of the amino acids.

Table 1. *Enantioselectivity Factor* α $(=\Delta F_D/\Delta F_L)$ *of the Guests* **1**–**5** *with the Hosts* **H-2** and **H-3**

	1	**2**	**3**	**4**	**5**
H-2	3.79	1.26	1.23	1.95	1.069
H-3	0.45	1.22	1.32	1.06	1.098

Enantioselectivity in fluorescence response may be explained by the formation of diastereoisomeric complexes of different stability involving the appended moiety, cyclodextrin, and the amino acid. To explain the stability of these complexes, association constants were calculated (*Table 2*) from the variation of the fluorescence intensities by using the *Benesi–Hildebrand* equation [8].

For a [guest]/[host] 1:1 (H/G 1:1) complex, the association constant K_s can be defined by *Eqn. 1* ($H + G \rightleftharpoons H \cdot G$). The numerical value of K_s can be obtained from the observed fluorescence decrease F_0/F as a function of CD concentration according to *Eqn. 2*, where F_{inf} is the fluorescence intensity when all of the guest molecules have been complexed with the host molecules. *Eqn. 2* assumes that only a 1:1 complex is formed. This assumption can be readily tested by using a reciprocal plot (*Benesi–Hildebrand* plot) of $\Delta F/F_0$ *vs.* 1/[H]. This plot will be linear if only a 1:1 complex is formed, but will show a curvature if complexes of other stoichiometry are formed [9]. *Fig. 2* shows the *Benesi–Hildebrand* plots for L-**4** (*Fig. 2,a*), D-**4** (*Fig. 2,b*), L-**5** (*Fig. 2,c*) and D-**5** (*Fig. 2,d*) with **H-2**. For these guests, the regression coefficients (R) are 0.987 and 0.999. For all the host · guest complexes, the R values are in the range of 0.97–0.99 indicating that a 1:1 complex is formed.

$$K_s = \frac{[H \cdot G]}{[H][G]} \tag{1}$$

$$1/(F_0 - F) = 1/(F_0 - F_{inf})\ [H]\ K_s + 1/(F_0 - F_{inf}) \tag{2}$$

The K_s values of the host · guest complexes are given in *Table 2*. Among all the amino acids studied, D-**3** exhibits the highest K_s value with **H-2**. It is also observed from the results that the D-enantiomers of all the amino acids (except for **1**) have higher K_s values indicating better recognition of the D-enantiomer by these hosts.

Conclusion. – The modified-cyclodextrin hosts **H-2** and **H-3** were applied in sensing studies of chiral amino acids showing enantioselective fluorescence quenching. The D-enantiomers of the amino acids are quenched more in the case of both hosts. The difference in fluorescence intensities, enantioselectivity factors, and association constants for all the guests may be due to the formation of complexes of different stability by weak forces, such as those involved in inclusion phenomena and in noncovalent interactions.

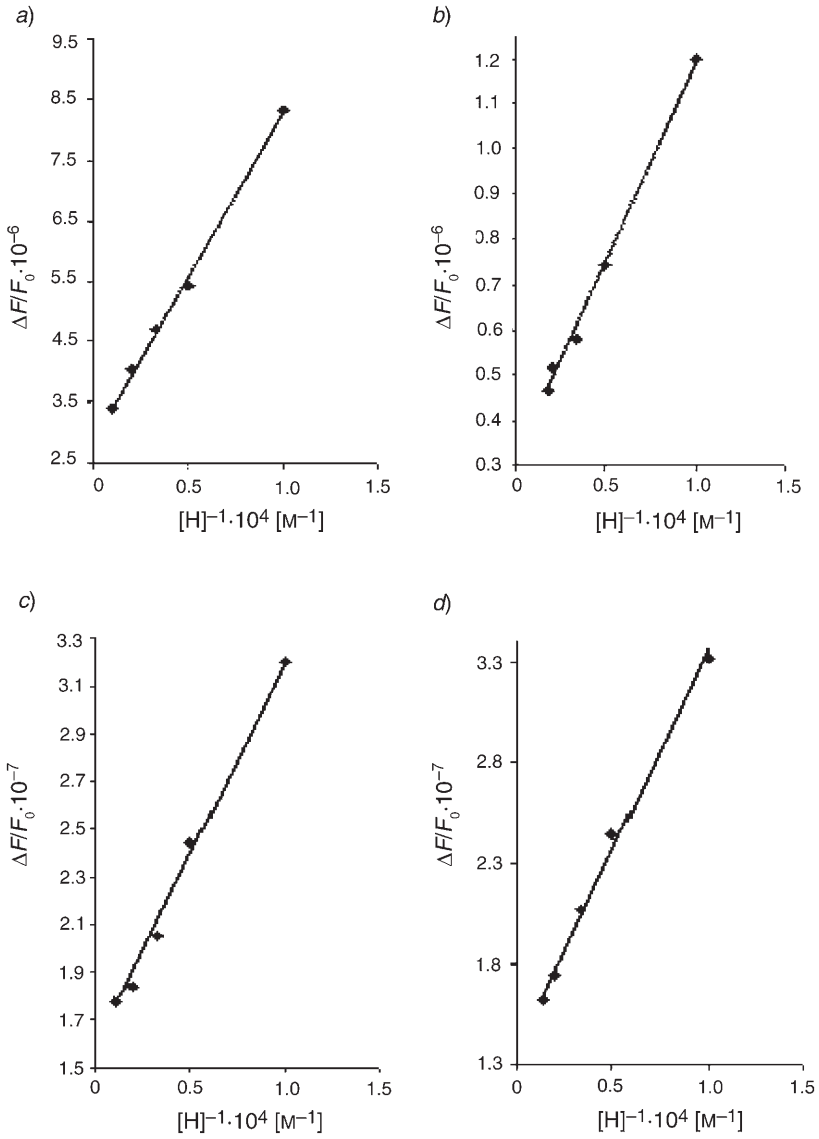


Fig. 2. Benesi–Hildebrand *plots for the estimation of association constants for* a) L-**4**, b) D-**4**, c) L-**5**, *and* d) D-**5** *with* **H-2**

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Table 2. *Association Constants* K_s [M^{-1}] *of the Amino Acids · Host Complexes*

	H-1	**H-2**	**H-3**
L-**1**	$1.2 \cdot 10^3 \pm 1 \cdot 10^2$	$4.0 \cdot 10^3 \pm 2 \cdot 10^2$	$2.0 \cdot 10^3 \pm 2 \cdot 10^2$
D-**1**	$1.2 \cdot 10^3 \pm 1 \cdot 10^2$	$3.3 \cdot 10^3 \pm 2 \cdot 10^2$	$1.8 \cdot 10^3 \pm 1 \cdot 10^2$
L-**2**	$2.0 \cdot 10^3 \pm 2 \cdot 10^2$	$2.5 \cdot 10^3 \pm 1 \cdot 10^2$	$5.0 \cdot 10^3 \pm 2 \cdot 10^2$
D-**2**	$2.0 \cdot 10^3 \pm 2 \cdot 10^2$	$6.6 \cdot 10^3 \pm 4 \cdot 10^2$	$1.0 \cdot 10^4 \pm 4 \cdot 10^2$
L-**3**	$1.0 \cdot 10^3 \pm 1 \cdot 10^2$	$2.2 \cdot 10^3 \pm 1 \cdot 10^2$	$4.5 \cdot 10^2 \pm 2 \cdot 10$
D-**3**	$1.0 \cdot 10^3 \pm 3 \cdot 10^2$	$3.3 \cdot 10^4 \pm 5 \cdot 10^2$	$4.2 \cdot 10^3 \pm 2 \cdot 10^2$
L-**4**	$3.5 \cdot 10^3 \pm 2 \cdot 10^2$	$1.0 \cdot 10^3 \pm 1 \cdot 10^2$	$6.0 \cdot 10^3 \pm 3 \cdot 10^2$
D-**4**	$3.5 \cdot 10^3 \pm 3 \cdot 10^2$	$1.0 \cdot 10^4 \pm 4 \cdot 10^2$	$7.5 \cdot 10^3 \pm 4 \cdot 10^2$
L-**5**	$1.0 \cdot 10^2 \pm 1 \cdot 10$	$1.5 \cdot 10^2 \pm 1 \cdot 10$	$1.2 \cdot 10^2 \pm 2 \cdot 10$
D-**5**	$1.0 \cdot 10^2 \pm 1 \cdot 10$	$1.0 \cdot 10^3 \pm 4 \cdot 10$	$2.5 \cdot 10^3 \pm 1 \cdot 10^2$

Experimental Part

General. Amino acids (*Aldrich*) were used as received without further purification. β-Cylodextrin (*Sigma Aldrich*) was recrystallized from H_2O and dried *in vacuo* at 60°. The hosts **H-2** and **H-3** were synthesized as reported by us earlier [5]. All solns. were prepared with high-purity H_2O. Stock solns. of amino acids ($1 \cdot 10^{-4}$ mol l^{-1}) and hosts ($1 \cdot 10^{-1}$ mol l^{-1}) were prepared by dissolving the appropriate amounts of the compounds in H_2O. UV Spectra: *Jasco* spectrophotometer; λ_{max} in nm. Fluorescence spectra: *Fluorolog* instrument, 1×0.2 cm quartz cell.

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