Fluorescence Sensing and Selective Binding of L- and D-Tryptophan-Modified Permethylated β-Cyclodextrins for Aliphatic Oligopeptides

Yu Liu*, Shu Kang, Yong Chen, Jun Shi and Chen-Feng Ke

Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P.R. China

Abstract: Two tryptophan-modified permethylated *β*-cyclodextrins, 6^{I}-L-Trp-6^{I}-deoxy- 2^{I},3^{I}-di-*O*-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl)-*β*-cyclodextrin (**3**) and 6^{I}-D-Trp- 6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl)-*β*-cyclodextrin (**4**), were synthesized, and their binding behaviors were investigated with the aliphatic oligopeptides, Leu-Gly, Gly-Leu, Gly-Pro, Glu-Glu, and Gly-Gly. Fluorescence spectrophotometric studies indicated that **3** and **4** can act as efficient fluorescence sensors for aliphatic oligopeptides. Due to their intermolecular co-inclusion binding mode with substrates, **3** and **4** not only afforded high binding constants of up to 10^{3}–10^{4} M^{-1} for guest oligopeptides but also good molecular selectivities of up to ca. 7 for Gly-Gly/Leu-Gly and Glu-Glu/Gly-Gly pairs.

Keywords: Cyclodextrin, oligopeptide, inclusion phenomena, synthetic receptors, host-guest modeling systems, fluorescence sensing, molecular recognition.

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides possessing hydrophobic cavities that are capable of binding a wide variety of organic and biological molecules *via* non-covalent interactions. Therefore, CDs are regarded as ideal prototypes for examining intermolecular interactions associated with molecular and chiral recognition [1-4]. During the past three decades, modified *β*-CDs and their methylated homologues have been widely utilized in separation sciences as important stereoselective selectors [5-8] and have potential applications as drug carriers [9]. Lipkowitz *et al.* studied the chiral recognitions of permethylated cyclodextrins (PMCDs), and gave an answer to the question, "how does PMCD work as chiral stationary phase in gas chromatography" [10-12]. That is, the binding site is the interior of the PMCD cavity, and the short-range dispersion force is the dominant intermolecular force responsible for host-guest coordination. Kano *et al.* reported the enantioselective complexation of PMCD with binaphthyls, in which hydrogen bonds were not dramatically involved [13]. Moreover, Botsi *et al.* reported NMR studies on the complexation process of CDs and PMCDs with (+)- and (–)-α-Pinene [14]. These studies have facilitated the understanding of the factors governing chiral recognition by CDs. On the other hand, grafting bio-active groups, such as *N*-dansylleucine and neuropeptide Leu-enkephalin groups, onto CDs may provide new vectors carrying signal molecules for targeting purposes [15-17].

Recently, we found that the introduction of tryptophan, pyridine-2,6-dicarboxamide, oxamido bis(2-benzoic) carboxyl, or a dithiobis(2-(benzoylamido) group onto β-CD could significantly enhance the binding ability and molecular selectivity of native β-CD towards aliphatic alcohols, steroids or oligopeptides [18-22]. However, to the best of our knowledge, the comparative studies on the molecular selective binding behaviors of a pair of diastereomeric CDs with enantiomeric sidearms have rarely been investigated [16], despite their importance in the understanding of the chiral and multiple recognition mechanisms of CDs. Herein, we report our investigation of the fluorescence sensing and molecular selective binding of a pair of L-/D-tryptophan-modified permethylated β-CDs (PMβCD, (Fig. **1**) for aliphatic oligopeptides (Fig. **2**) by means of fluorescence and 2D NMR spectroscopy. It is our special interest to examine the molecular recognition mechanism of oligopeptides by molecular receptors bearing chiral centers, which will serve our further understanding of this recently developing but less investigated area of supramolecular chemistry.

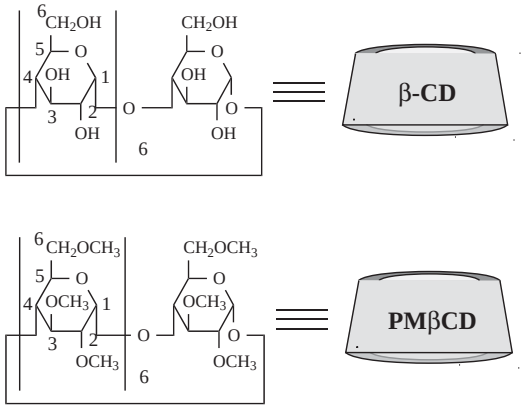
MATERIALS AND METHODS

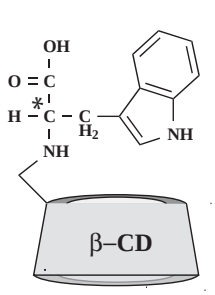
Materials

Reagent grade *β*-CD (Huaxing Biochemistry, China) was recrystallized twice from water and dried in vacuum at 95ºC for 24 h prior to use. L/D-tryptophan and the L-amino acid containing peptides Leu-Gly, Gly-Leu, Gly-Pro, Glu-Glu, and Gly-Gly (> 98% purity) were obtained from Tokyo Peptide Institute (Japan) and used without further purification. The isoelectric pKa values for each peptide standard were 5.97, 5.98, 5.97, 5.80, and 5.93 for $pI_{(Leu\text{-}Gly)}$, $pI_{(Gly\text{-}Leu)}$, $pI_{(Gly\text{-}Gly)}$, $pI_{(Gly\text{-}Pro)}$, and $pI_{(Glu\text{-}Glu)}$, respectivelye. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 48 h and then distilled under reduced pressure prior to use. Acetonitrile (CH_3CN) was dried over calcium hydride and then distilled fractionally to give the anhydrous solvent. Mono[6-*O*-(*p*-toluenesulfonyl)]-β-CD (6-OTs-β-CD) was prepared by the reaction of *p*-toluenesulfonyl chloride with β-CD in alkaline aqueous solution [23]. Mono(6-(*p*-tolysulfonyl)) permethylated β-CD was prepared by the direct methylation of tosylated CD according to the literature [24]. 6-L-Trp-β-CD (**1**) and 6-D-Trp-β-CD (**2**) were synthesized according to our previous report [19]. Disodium

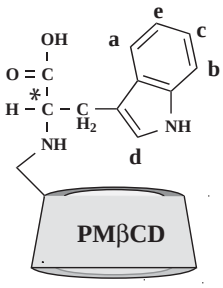
*Address correspondence to this author at the Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P.R. China; Tel: 86-022-23503625; Fax: 86-022-23503625; E-mail: yuliu@nankai.edu.cn

1386-2073/07 $50.00+.00





1. L-Trp; **2**. D-Trp



3. L-Trp; **4**. D-Trp

Fig. (1). Molecular structures of hosts **1-4**.

hydrogen phosphate dodecahydrate (25.79 g) and sodium dihydrogen phosphate dihydrate (4.37 g) were dissolved in distilled, deionized water (1 L) to make a 0.1 M phosphate buffer solution of pH 7.20 for spectral analysis.

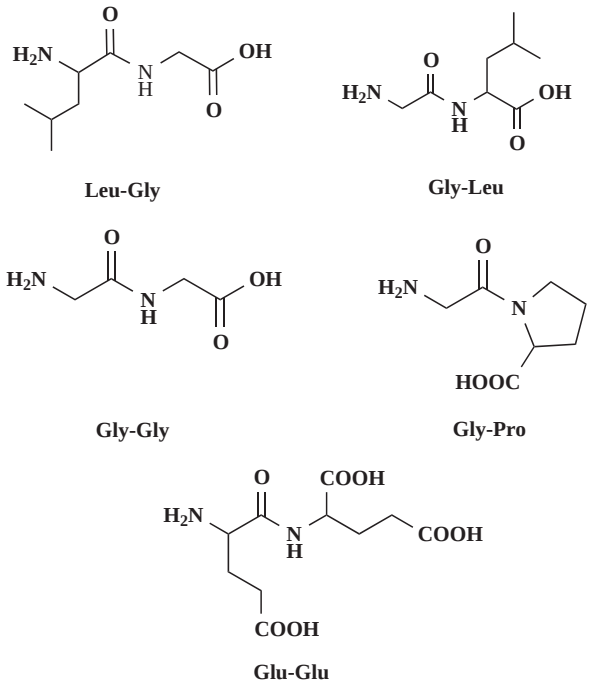


Fig. (2). Molecular structures of guest oligopeptides.

Instruments

Fluorescence spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) at 25°C on a JASCO FP-750 fluorescence spectrometer with excitation and emission slits of 10 nm width. Optical rotation was measured in a quartz cell (light path 10 cm) at 20°C on a PerkinElmer Model 341 polarimeter. Elemental analysis was performed on a Perkin-Elmer 2400C instrument, and NMR spectra were obtained using a Varian Mercury VX300 spectrometer. Fluorescence lifetimes were recorded on a FLS920 Combined Steady State and Lifetime Spectrometer (Edinburgh Instruments) with a time resolution of 0.19 ns. A nanosecond pulsed flash lamp (NF900) filled with hydrogen gas was employed as a pulsed light source. Maximum counts of up to 10 000 were collected for each measurement.

Synthesis of 6^{I}-L-Trp-6^{I}-Deoxy-2^{I},3^{I}-Di-O-Methyl-Hexakis($2^{II\text{-}VII}$,$3^{II\text{-}VII}$,$6^{II\text{-}VII}$-Tri-*O*- Methyl)-*β*-CD (3)

L-Tryptophan (1.0 g, 4.9 mmol), K_2CO_3 (0.7 g, 5.0 mmol) and mono(6-(*p*-tolysulfonyl))permethylated β-CD (2.0 g, 1.2 mmol) were mixed in anhydrous CH_3CN (20 mL), and the resulting mixture was heated to reflux for 2 days with stirring under a nitrogen atmosphere. After cooling to room temperature, the precipitate was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in alcohol and purified by chromatography over silica gel using ethyl acetate-petroleum ether (1:2.5, v/v) as eluent to afford **3** as a brown crystal: (45.3%): UV-Vis λ_{max} (H_2O)/nm (log ε) 220 nm (4.11), 260 nm (3.43); $[\alpha]_D^{20}$ = +137.5; ^{1}H NMR (300 MHz, D_2O, ppm) δ: 7.67 (d, *J* = 5.4 Hz, 1H), 7.51-7.45 (m, 3H), 7.18 (d, *J* = 6.0 Hz, 1H), 5.23-4.92 (m, 7H, H-1), 4.05-3.06 (m, 105H), of which 3.85 (m, 1H, H_f of Trp), 3.42 (s, 21H, 3-OMe), 3.33 (s, 21H, 2-OMe), 3.20 (s, 18H, 6-OMe). ^{13}C NMR(300 MHz, $CDCl_3$, ppm) δ_C 181.3, 144.7, 129.9, 127.9, 117.4, 99.1, 99.04, 98.98, 98.8, 82.1, 81.95, 81.88, 81.82, 81.73,81.62,80.31, 77.67, 77.64, 77.44, 77.02, 76.6, 71.45, 71.34, 71.3, 71.24, 71.14, 71.08, 70.96, 61.68, 61.56, 61.44, 61.33, 61.29, 59.12, 59.07, 59.01, 58.97, 58.52, 38.4, 22.6, 21.7. Positive ion electrospray MS, *m/z* 1601.61, $M^{+\cdot}$; Anal. Calcd. for $C_{73}H_{120}O_{36}N_2$: C 54.74; H 7.55, N 1.75%. found: C 54.51, H 7.25, N 1.84%.

Synthesis of 6^{I}-D-Trp-6^{I}-Deoxy-2^{I},3^{I}-Di-O-Methyl-Hexakis($2^{II\text{-}VII}$,$3^{II\text{-}VII}$,$6^{II\text{-}VII}$-Tri-*O*- Methyl)-*β*-CD (4)

Compound **4** was prepared from D-tryptophan and mono(6-(*p*-tolysulfonyl)) permethylated *β*-CD in 28.6% yield according to a procedure similar to that in the synthesis of **3** (Fig. **3**). UV-Vis λ_{max} (H_2O)/nm (log ε) 220 nm (3.91), 260 nm (3.25); $[\alpha]_D^{20}$ = +132; ^{1}H NMR (300MHz, D_2O, ppm) δ: 7.63 (d, *J* = 9 Hz, 1H) 7.47-7.41(m, 3H), 7.10 (d, *J* = 6 Hz, 1H), 5.29-4.98 (m, 7H, H-1), 3.80-3.11 (m, 105H), of which 3.85 (m,1H, H_f of Trp), 3.38 (s, 21H, 3-OMe), 3.29 (s, 21H, 2-OMe), 3.16 (s, 18H, 6-OMe). ^{13}C NMR(300 MHz, $CDCl_3$, ppm) δ_C 181.3, 144.7, 129.9, 127.9, 117.4, 99.1, 99.04, 98.98, 98.8, 82.1, 81.95, 81.88, 81.82, 81.73,81.62,80.31, 77.67, 77.64, 77.44, 77.02, 76.6, 71.45, 71.34, 71.3, 71.24, 71.14, 71.08, 70.96, 61.68, 61.56, 61.44, 61.33, 61.29, 59.12, 59.07, 59.01, 58.97, 58.52, 38.4, 22.6, 21.7. Negative ion electrospray MS, *m/z* 1601.67 [$M^{-\cdot}$]; Anal. Calcd. for $C_{73}H_{120}O_{36}N_2$: C 54.74, H 7.55, N 1.75%. found: C 54.62, H 7.51, N 2.01%.

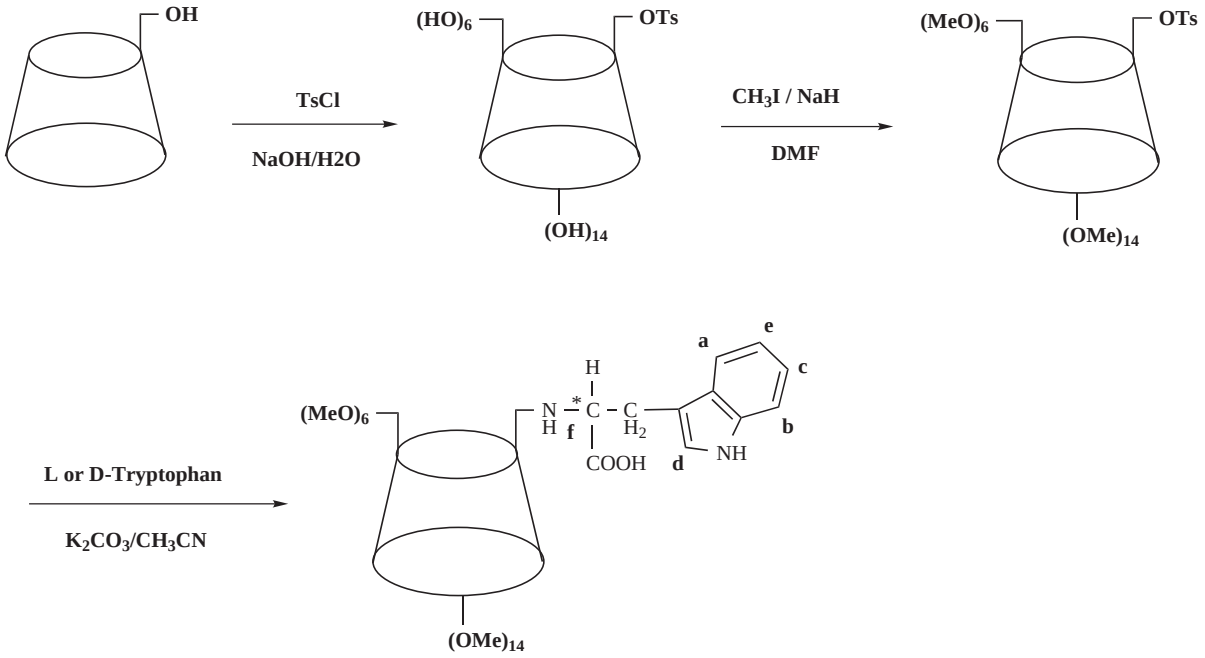


Fig. (3). Synthetic routes for hosts **3**-**4**.

RESULTS AND DISCUSSION

Conformation Analysis

The conformations of hosts **3** and **4** were investigated by using 2D NMR spectroscopy. It is well documented that two protons located closely in space (generally 4 Å or less) can produce a cross-peak between the relevant protons in the NOESY or ROESY spectra [25]. As shown in Fig. **4**, the ROESY spectrum of host **4** in D_2O displays a clear cross-peak signals assigned to the NOE (Nuclear Overhauser Effect) correlations between the interior protons of the PMβCD cavity and the protons of the tryptophan substituent (peaks A, B, and D) as well as the intramolecular correlations among the tryptophan protons (peak C). Among these peaks, the cross-peaks A and B were assigned to the NOE correlations between the H3 protons of PMβCD cavity and the H_a, H_c, H_d, and H_e protons of the indole group of the tryptophan substituent. The cross-peak D was assigned to the NOE correlations between the H3 protons of PMβCD cavity and the H_f proton of tryptophan. Because the H5/H6 protons were located near the narrow side of the PMCD cavity but the H3 protons were located near the wide side, these NOE correlations indicate that the tryptophan group was included in the PMβCD cavity from the wide side. Similar NOE correlations were also observed in the ROESY spectrum of host **3**. Because the tryptophan group was linked at the narrow side of the PMβCD cavity of **3** and **4** (Fig. **1**), we deduced that hosts **3** and **4** may adopt an intermolecularly included conformation as illustrated in Fig. **4c**. That is, the tryptophan group was accommodated in the PMβCD cavity of another Trp-PM*β*CD to form the head-to-tail dimer.

Fluorescence Sensing and Binding Mode

Many researches have demonstrated that, upon inclusion complexation with a guest molecule, the fluorescence emission of fluorophore-appended mono-CDs is either quenched as a consequence of decomplexation of the initially self-included fluorophore moiety [18,19, 26-28] or increased due to an intramolecular co-inclusion complexation [29]. In our studies, both L-Trp-PMβCD **3** and D-Trp-PMβCD **4** exhibited enhanced fluorescence upon the gradual addition of aliphatic oligopeptides as shown in Fig. **5**. These unique fluorescence behaviors may enable **3** and **4** to be efficient fluorescence sensors for optically inserted molecules.

A possible reason for the enhanced fluorescence of **3** and **4** may be the increased microenviromental hydrophobicity and/or steric shielding around the tryptophan fluorophore upon inclusion complexation, and this hypothesis is supported by the 2D NMR measurements. As illustrated in Fig. **6**, the ROESY spectrum of an equimolar mixture of **4** and Glu-Glu displayed clear NOE correlations (peaks A) between the H3 protons of PMβCD and H_a/H_c/H_d/H_e protons of tryptophan, indicating that the indolyl group of tryptophan substituent was still located in the PMβCD cavity. Moreover, the NOE correlations (peak B) between the H5 protons of PMβCD and the H_p protons of Glu-Glu as well as the NOE correlations (peak C) between the H3 protons of PMβCD and the H_r protons of Glu-Glu jointly indicated that the guest oligopeptide was included in the PMβCD cavity from the narrow side. In addition, Fig. **6** shows the NOE correlations (peak E) between H_r protons of Glu-Glu and H_c protons of the indolyl group of the tryptophan substituent, indicating the close location of the indolyl moiety and guest Glu-Glu. According to these NOE correlations, we deduced a possible binding mode of **4** with Glu-Glu (Fig. **6d**), where the Trp group of **4** and Glu-Glu are both included in the PMβCD cavity. This binding mode consequently rationalizes the enhanced fluorescence of **4** in the presence of the guest oligopeptide. Firstly, the inclusion of an oligopeptide can extrude water from the PMβCD cavity and thus make the

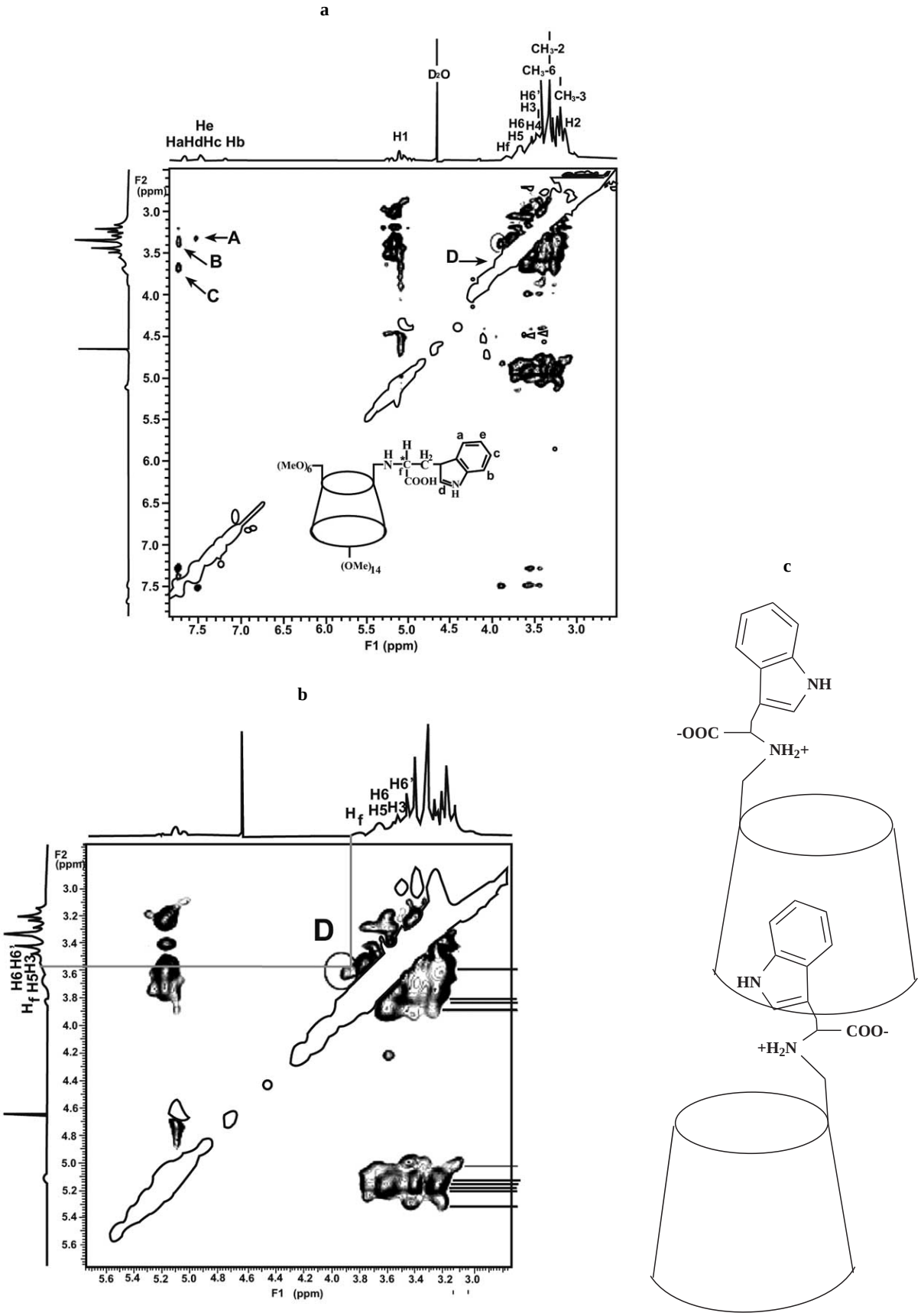


Fig. (4). (**a**) ROESY spectrum and (**b**) partial ROESY spectrum of **4** (2.0 mM) in D_2O with a mixing time of 270 ms at 298.1K. (**c**) Possible conformation of **4**.

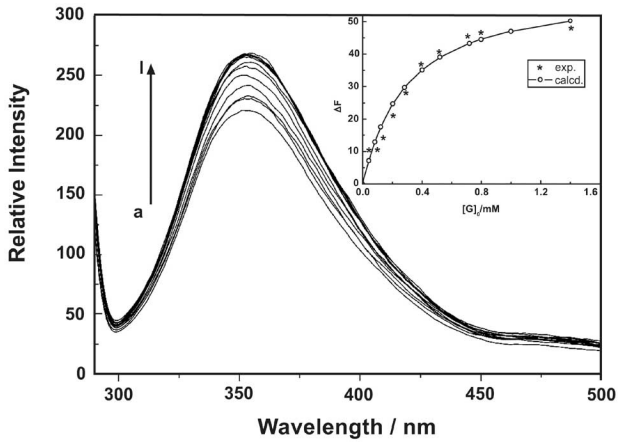


Fig. (5). Fluorescence spectral changes of host **4** (2.5×10^{-5} M) upon addition of Gly-Leu (0 to 1.60×10^{-3} M from a to l) in phosphate buffer solution at 298.1 K, and the nonlinear least-square analysis (inset) of the differential intensity (ΔF) to calculate the complex stability constant (K_s).

cavity more hydrophobic. Secondly, the tryptophan fluorophore can be efficiently shielded from deactivating water attack by the guest oligopeptide that is located nearby. As a joint result of these two factors, **3** and **4** give enhanced fluorescence upon inclusion complexation with the oligopeptide.

This substituent/adjacent host/guest co-inclusion mode was also confirmed by fluorescence and fluorescence lifetime experiments. As a good reference system, the Trp/PMβCD mixture emits stronger fluorescence than the free Trp, indicating that the Trp is included in the PMβCD cavity. With the addition of guest oligopeptide, the fluorescence of the Trp/PMβCD mixture was further enhanced, which was similar to the fluorescence behaviors of hosts **3**-**4** upon complexation with guest oligopeptides (Fig. **7**). These results are in good agreement with the proposed co-inclusion mode. In this mode, the included guest oligopeptide prevents the closely located tryptophan fluorophore from attack by deactivating water, which consequently leads to enhanced fluorescence. Moreover, the fluorescence lifetimes were also measured to investigate the microenvironmental hydrophobicity around the Trp fluorophores in **3** and **4**. As can be seen in Table **1**, both **3** and **4** gave shorter lifetimes and longer lifetimes. These results indicate that the Trp fluorophore may have been located in two sorts of environments of distinctly different hydrophobicity and also that the interconversion of these two species was much slower than the fluorescence decay, which occurs on the nanosecond time scale. Thus, the shorter lifetimes (τ_S) and the longer lifetimes (τ_L) are reasonably assigned to those Trp fluorophores that are exposed to the bulk aqueous solution and located in the PMβCD cavity, respectively. Interestingly, the τ_L of **3** (τ_L = 10.2 ns) or **4** (τ_L = 10.8 ns) is higher than that of **1** (τ_L = 9.4 ns) or **2** (τ_L = 9.3 ns) [19], indicating that the PMβCD cavity can provide a more hydrophobic microenvironment for the Trp fluorophore than the native CD cavity to some extent. Moreover, by comparing the lifetimes of **3**-**4** in the absence and presence of guest oligopeptides, we find that the τ_L of **3** or **4** is almost unchanged, but the Φ_L appreciably increases, after the addition of guest oligopeptides, which indicates that the Trp fluorophore is not excluded from the PMβCD cavity by guest oligopeptides. Therefore, the results of fluorescence and fluorescence lifetime experiments jointly demonstrate the co-inclusion mode of **3**-**4** with guest oligopeptides.

Binding Ability and Molecular Selectivity

Assuming a 1:1 host-guest stoichiometry that most of the mono-modified CDs adopt upon complexation with guest molecules, the complex stability constant (K_s) can be calculated using a nonlinear least squares curve-fitting method [12]. For each host-guest combination, good fits between the experimental and calculated data were observed. In repeated measurements, the K_s values were reproducible within an error of ±5%. The complex stability constants (K_s) and Gibbs free energy changes ($-\Delta G^o$) obtained for the complexation of **1**-**4** with oligopeptides are listed in Table **2**.

As can be seen in Table **2**, Trp-PMβCDs **3** and **4** showed larger complex stability constants with all of the oligopeptide guests than their unmethylated homologues **1** and **2**, which indicates that permethylation can efficiently improve the binding abilities of β-CDs. A possible explanation is that the introduction of multiple methyl groups destroys the original hydrogen bond network of β-CD and thus makes the β-CD cavity more hydrophobic. Moreover, the enhanced flexibility resulting from the permethylation of β-CD also makes the β-CD cavity more suitable for the inclusion of guest molecules. These two factors lead to the enhanced hydrophobic interactions between PMβCD and the accommodated guest. Moreover, we have demonstrated that the Trp-PMCDs **3** and **4** adopt an intermolecular co-inclusion binding mode upon complexation with oligopeptides, where the tryptophan group and the guest oligopeptide are both included in a PMβCD cavity. In a neutral environment, the carboxylic groups of guest oligopeptides mainly exist in the COO^- form, and the amino groups are partially protonated. Therefore, the electrostatic interactions between the charged tryptophan group of the host and the COO^- as well as NH_3^+ groups of guest oligopeptide consequently strengthen the host-guest binding. In addition, the hydrogen bonds between the tryptophan group and the guest oligopeptide also contribute to the strong binding of Trp-PMβCDs. Through the combination of these factors, Trp-PMβCDs show stronger binding abilities than their unmethylated homologues.

It is also interesting to compare the molecular selectivity of **3** and **4**. For **3**, the K_s values varied in an order of Gly-Gly > Gly-Pro > Gly-Leu > Glu-Glu > Leu-Gly, but this order changed to Glu-Glu > Leu-Gly > Gly-Pro > Gly-Leu > Gly-Gly for **4**. That is, host **3** displayed stronger binding towards the non chiral dipeptide Gly-Gly, while the enantiomeric (diastereisomeric) dipeptides Leu-Gly, Gly-Leu, Gly-Pro, and Glu-Glu were bound better by host **4**. This phenomenon may be attributed to the different location of the L-/D-tryptophan groups in Trp-PMβCDs relative to the rim of the PMβCD cavity. Possessing a D-Trp substituent, **4** may adopt a conformation with the amino group of tryptophan located close to the PMβCD rim but the caroboxylic group distant from the PMβCD rim. Therefore, D-Trp-modified **4** showed the strongest binding for Glu-Glu, containing three carboxylic

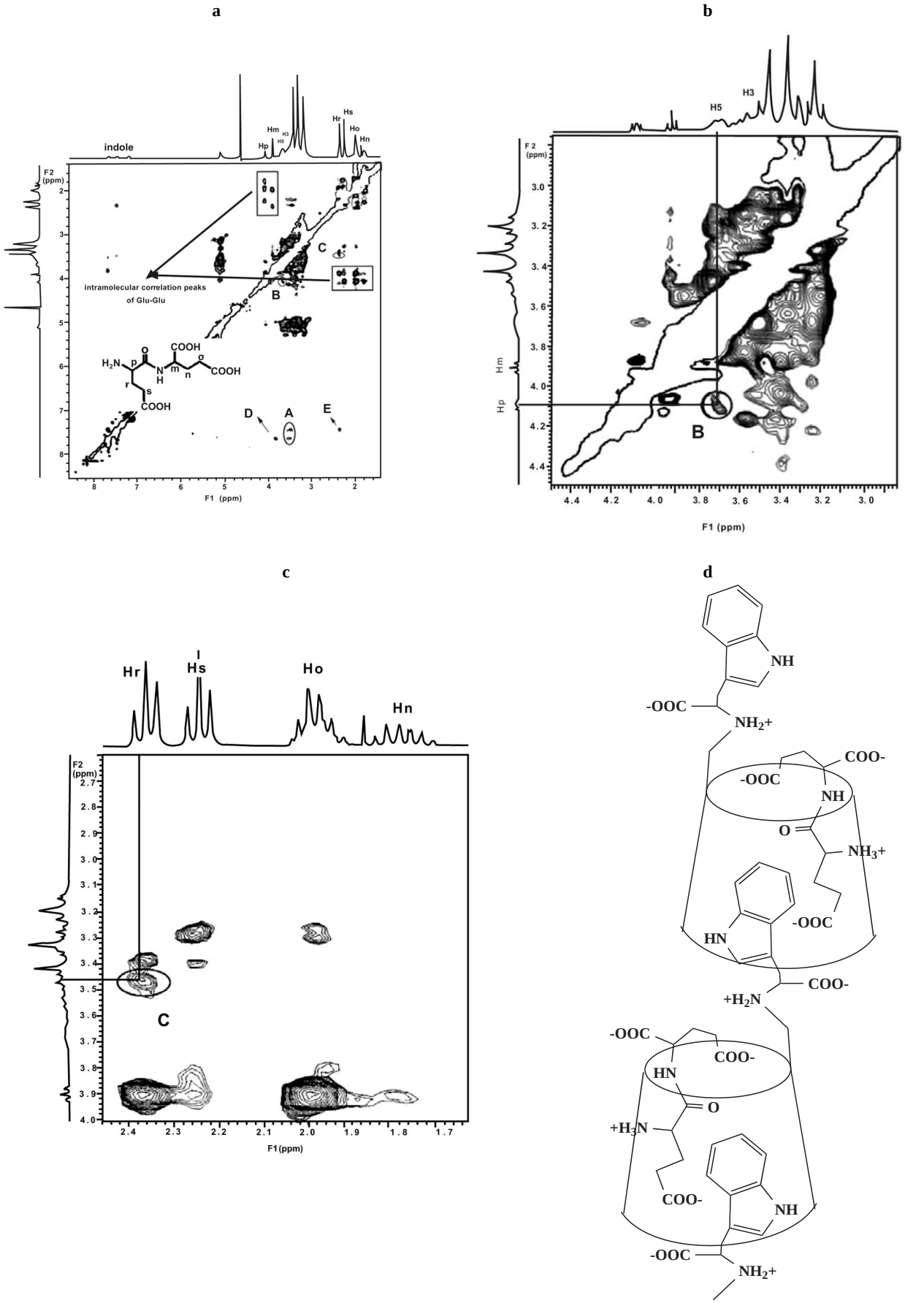


Fig. (6). (**a**) ROESY spectrum and (**b**,**c**) partial ROESY spectra of an equimolar mixture of **4** with Glu-Glu (2.0 mM each) in D_2O with a mixing time of 250 ms at 298.1 K; (**d**) the possible binding mode of **4** with Glu-Glu.

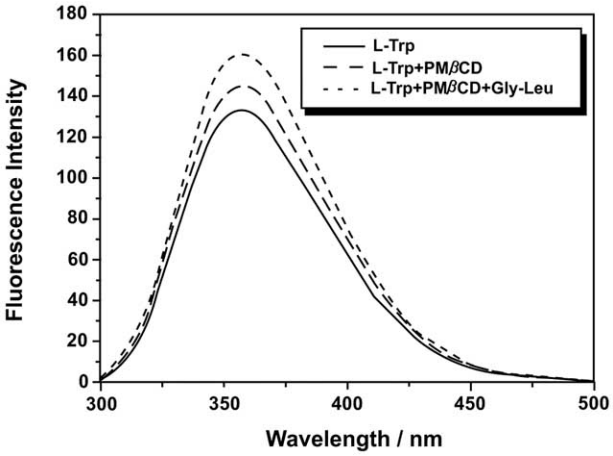


Fig. (7). Fluorescence spectral changes of L-Trp (2.5×10^{-5} M) upon addition of excess PMβCD (5.0×10^{-4} M) and Gly-Leu (5.0×10^{-4} M) in phosphate buffer solution (pH 7.20) at 25°C.

Table 1. Fluorescence Lifetimes and Relative Quantum Yields of 3 and 4 (2.0×10^{-5} M) in the Absence and Presence of Guest Oligopeptides (1.6×10^{-3} M) in Aqueous Phosphate Buffer Solution (pH 7.20) at 25°C

Host	Guest	τ_S (ns)	Φ_S (%)	τ_L (ns)	Φ_L (%)
3		2.7	43	10.2	57
3	Gly-Leu	2.8	42	9.9	58
3	Leu-Gly	2.8	42	10.1	58
4		3.1	52	10.8	48
4	Gly-Leu	3.1	48	10.6	52
4	Leu-Gly	3.0	48	10.6	52

groups, through the electrostatic attractions between the NH_2^+ group of tryptophan and the COO^- groups of Glu-Glu. On the other hand, L-Trp-modified **3** may adopt an opposite conformation to that of **4**, where the carboxylic group of tryptophan was located close to the PMβCD rim but the amino group was distant from the PMβCD rim. Therefore, **3** showed higher binding affinity for the guest oligopeptides containing a Gly-NH_2 terminus (Gly-Gly, Gly-Pro, Gly-Leu) through electrostatic attractions between the Gly-NH_3^+ group of the guest oligopeptide and the COO^- group of **3**. Moreover, the chiral fit relationship between host and guest may also contribute to the different selectivities of **3** and **4**. For **4**, the introduction of a D-Trp residue to the PMβCD, made up of seven D-glucose units linked by α-1,4-glucose bonds, enhanced the chiral character of the PMβCD cavity, which consequently resulted to a better chiral fit between **4** and the selected enantiomeric (diastereisomeric) dipeptides containing L-amino acid residues. In contrast, the introduction of a L-Trp residue lead to the partial loss of chiral character of the PMβCD cavity in **3**. This loss of chiral character did not favor the chiral fit of **3** with the enantiomeric (diastereisomeric) dipeptides, but instead favored its chiral fit with the non-chiral dipeptide Gly-Gly to some extent. As a combination of these factors, **3** and **4** exhibited good molecular selectivities for Gly-Gly/Leu-Gly ($K_{s\mathbf{3}/\text{Gly-Gly}}/K_{s\mathbf{3}/\text{Leu-Gly}} = 6.8$) and Glu-Glu/Gly-Gly ($K_{s\mathbf{4}/\text{Glu-Glu}}/K_{s\mathbf{4}/\text{Gly-Gly}} = 7.0$) pairs, respectively.

Table 2. Complex Stability Constants (K_s) and Gibbs Free-Energy Changes (-Δ*G*°) for the Inclusion Complexation of Oligopeptides by Hosts 1-4 in Buffer Solution at 25°C

Guest	Host	K_s (M^{-1})	log K_s	-Δ*G*° (kJ/mol)
Gly-Gly	**1**	a	–	–
	2	a	–	–
	3	9090 ± 120	3.96	22.59
	4	1480 ± 70	3.17	18.10
Gly-Pro	**1**	a	–	–
	2	a	–	–
	3	8160 ± 200	3.91	22.33
	4	6150 ± 110	3.79	21.63
Glu-Glu	**1**	a	–	–
	2	a	–	–
	3	2030 ± 80	3.31	18.88
	4	10390 ± 180	4.02	22.93
Gly-Leu	**1**	363 ± 20	2.56	14.60
	2	503 ± 25	2.70	15.42
	3	5110 ± 140	3.71	21.17
	4	3660 ± 100	3.56	20.34
Leu-Gly	**1**	1020 ± 30	3.01	17.17
	2	610 ± 12	2.78	15.90
	3	1330 ± 25	3.12	17.83
	4	6790 ± 130	3.83	21.87

a: The spectral changes were too weak to calculate the K_s value.

CONCLUSIONS

In the present investigation, we have demonstrated that L- and D-Trp-PMβCDs can be used not only as efficient fluorescence sensors for the molecular recognition of optically active aliphatic oligopeptides but also as a convenient and powerful model of molecular receptors for enhancing their guest binding ability and selectivity. Furthermore, the intermolecular co-inclusion binding mode observed may share some similarities with biological molecular recognition involving the multicomponent, induced-fit receptor-substrate interactions.

ACKNOWLEDGEMENTS

We are grateful to 973 Program (2006CB932900), NNSFC (No. 90306009, 20402008, 20421202, and 20572052), Special Fund for Doctoral Program from the Ministry of Education of China (20050055004), and Tianjin Natural Science Foundation (06YFJMJC04400) for financial support.

ABBREVIATIONS

CD = Cyclodextrin

6-OTs-β-CD = Mono[6-*O*-(*p*-toluenesulfonyl)]-β-CD

PMCD = Permethylated cyclodextrin

PMβCD = Permethylated β-CD

REFERENCES

[1] Schneider, H.-J. *Agnew. Chem. Int. Ed.*, **1991**, *30*, 1417-1436.

[2] Harata, K. *Chem. Rev.*, **1998**, *98*, 1803-1827.

[3] Szejtli, J.; Osa, T. *Cyclodextrins* In: *Comprehensive Supramolecular Chemistry*, Eds: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, *Elsevier: Ixfird*, **1996;** Vol. *3*.

[4] Easton, C.J.; Lincoln, S.F. *Chem. Soc. Rev.*, **1996**, 163-170.

[5] Armstrong, D.W.; Ward, T.J.; Armstrong, R.D.; Beesley, T.E. *Science*, **1996**, *232*, 1132-1135.

[6] Hinze, W.L.; Riehl, T.E.; Armstrong, D.W.; Semond, M.; Alak, A.; Ward, T. *Anal. Chem.*, **1985**, *57*, 237-242.

[7] Daffe, V.; Fastrez, J. *J. Chem. Soc., Perkin Trans. 2*, **1983**, 789-796.

[8] Pirkle, W.H.; Hyun, M.H.; Bank, B. *J. Chromatogr. A*, **1984**, *316*, 585-604.

[9] Uekama, K.; Hirayama, F.; Irie T. *Chem. Rev.*, **1998**, *98*, 2045-2076.

[10] Lipkowitz, K.B.; Pearl, G.; Coner, B.; Peterson, M.A. *J. Am. Chem. Soc.*, **1997**, *119*, 600-610.

[11] Lipkowitz, K.B.; Coner, R.; Peterson, M.A. *J. Am. Chem. Soc.*, **1997**, *119*, 11269-11276.

[12] Lipkowitz, K.B.; Coner, R.; Peterson, M.A.; Morreale, A.; Shackelford, J. *J. Org. Chem.*, **1998**, *63*, 732-745.

[13] Kano, K.; Yoshiyasu, K.; Hashimoto, S. *J. Chem. Soc. Chem. Commun.*, **1989**, 1278-1279.

[14] Botsi, A.; Perly, B.; Hadjoudis E. *J. Chem. Soc. Perkin Trans. 2*, **1997**, 89-94.

[15] Parrot-Lopez, H.; Djedaini, F.; Perly, B.; Coleman, A.W.; Galons, H.; Miocque, M. *Tetrahedron Lett.*, **1990**, *31*, 1999-2002.

[16] Ikeda, H.; Nakamura, M.; Ise, N.; Oguma, N.; Nakamura, A.; Ikeda, T.; Toda, F.; Ueno A. *J. Am. Chem. Soc.*, **1996**, *118*, 10980-10988.

[17] Djedaini-Pilard, F.; Desalos, J.; Perly, B. *Tetrahedron Lett.*, **1993**, *34*, 2457-2460.

[18] Liu, Y.; Han, B.-H.; Sun, S.-X.; Wada, T.; Inoue, Y. *J. Org. Chem.*, **1999**, *64*, 1487-1493.

[19] Wang, H.; Cao, R.; Ke, C.-F.; Liu, Y.; Wada, T.; Inoue, Y. *J. Org. Chem.*, **2005**, *70*, 8703-8711.

[20] Liu, Y.; Yang, Y.-W.; Song, Y.; Zhang, H.-Y.; Ding, F.; Wada, T.; Inoue, Y. *ChemBioChem.*, **2004**, *5*, 868-871.

[21] Liu, Y.; Zhao, Y.-L.; Chen, Y.; Ding, F.; Chen, G.-S. *Bioconjugate Chem.*, **2004**, *15*, 1236-1245.

[22] Liu, Y.; Chen, G.-S.; Chen, Y.; Ding, F.; Liu, T.; Zhao, Y.-L. *Bioconjugate Chem.*, **2004**, *15*, 300-306.

[23] Petter, R.C.; Salek, J. S.; Sikorski, C. T.; Kumaravel, G.; Lin, F.-T. *J. Am. Chem. Soc.*, **1990**, *112*, 3860-3868.

[24] Lai, X.H.; Ng, S.C. *Tetrahedron Lett.*, **2004**, *45*, 4469-4472.

[25] Schneider, H.-J.; Hacket, F.; Rudiger, V.; Ikeda, H. *Chem. Rev.*, **1998**, *98*, 1755-1785.

[26] Wallimann, P.; Marti, T.; Furer, A.; Diederich, F. *Chem. Rev.*, **1997**, *97*, 1567-1608.

[27] Nakamura, M.; Ikeda, A.; Ise, N.; Ikeda, H.; Toda, F.; Ueno, A. *J. Chem. Soc. Chem. Commun.*, **1995**, 721-722.

[28] Ikeda, H.; Nakamura, M.; Ise, N.; Toda, F.; Ueno A. *J. Org. Chem.*, **1997**, *62*, 1411-1418.

[29] Narita, M.; Hamada, F.; Suzuki, I.; Osa, T. *J. Chem. Soc., Perkin Trans. 2*, **1998**, 2751-2758.

Received: March 28, 2007 **Revised: April 11, 2007** **Accepted: April 19, 2007**