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Synthesis of ethylenediamine linked β-cyclodextrin dimer and its analytical application for tranilast determination by spectrofluorimetry

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Abstract—A synthesis of β-cyclodextrin (β-CD) dimer, containing two β-CD moieties that are linked through their sides by ethylenediamine, was presented. The dimer was characterized by means of IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. The inclusion complexation behavior of β-cyclodextrin dimer with tranilast was studied in an aqueous KH_2PO_4–citric acid buffer solution of pH 2.00 at room temperature by spectrofluorimetry. Based on the significant enhancement of fluorescence intensity of tranilast, a spectrofluorimetric method with high sensitivity and selectivity was developed for the determination of tranilast in bulk aqueous solution in the presence of ethylenediamine β-CD dimer. The apparent association constant of the complex was 8.39×10^3 L mol^{-1}, and the linear range was $10.8–1.40 \times 10^4$ ng mL^{-1} with the detection limit 3.2 ng mL^{-1}. There was no interference from the excipients normally used in tablets and serum constituents. The proposed method was successfully applied to the determination of tranilast in serum.

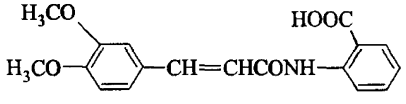
1. Introduction

Cyclodextrins (CDs), the cyclic oligosaccharides consisting of six or more D-(+)-glucopyranose units, are well known to accommodate various guest molecules into their truncated cone-shaped hydrophobic cavity in aqueous solution.[1–3] This fascinating property enables them to be successfully used as drug carriers,[4,5] separation reagents, [6] enzyme mimics,[7] photochemical sensors,[8,9] catalysis,[10] host–guest interactions[11], and molecular recognitions.[12] However, the apparent association constants of natural CDs and their simple derivatives with model substrates are generally 10^2 L mol^{-1}, and this limits their application as enzyme mimics and as antibody mimics to a great extent. For application in molecular recognition and chemical sensing, methods for selectively and efficiently modifying CDs are highly desirable.

Cyclodextrin dimers tethered by the spacer (or linker) of different sizes and shapes may afford distinctly different apparent association abilities and molecular selectivities. Hence, diverse functional groups such as alkanedioates,[13,14] disulfides,[15,16] dipyridine[17], and imidazole[18,19] have been used as the linker between two cyclodextrin units. Unexpectedly, their molecular recognition behaviors have not been extensively investigated.

In this paper, we reported the synthesis of ethylenediamine linked β-CD dimer[20] by the reaction of 6-OTs-β-CD with ethylenediamine, whose solubility greatly enhanced compared to other alkanedioate linked β-CD dimers, and it was characterized by means of IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. The synthesis of ethylenediamine linked β-CD dimer was easy to carry out and proceeds with satisfactory yield.

Tranilast, chemically *N*-(3,4-dimethoxycinnamoyl) anthranilic acid (Scheme 1), is an anti-allergic drug for



Scheme 1. The structure of tranilast.

Keywords: Synthesis; β-Cyclodextrin dimer; Ethylenediamine; Tranilast; Spectrofluorimetry.
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the treatment of allergic asthma and other allergic diseases. At present, the analytical techniques that have been applied to the determination of tranilast were HPLC [21,22] and reversed-phase high performance liquid chromatography.[23] Most of these methods used organic solvent, needed expensive instruments, required strictly controlled reaction conditions, and involved time-consuming operating steps. Therefore, we need to develop a simple, highly sensitive, and selective method such as spectrofluorimetry for the determination of tranilast.

Tranilast could give off fluorescence in organic solvent, but it showed a yield in the aqueous solution. When ethylenediamine linked β-CD dimer was added to the aqueous solution of tranilast, ethylenediamine linked β-CD dimer reacted with tranilast to form an inclusion complex, we could observe an obvious increase in fluorescence intensity of tranilast. However, the fluorescence intensity did not obviously enhance when β-CD was added to the aqueous solution of tranilast. So the spectrofluorimetric study of the interaction between ethyl enediamine linked β-CD dimer and tranilast was carried out.

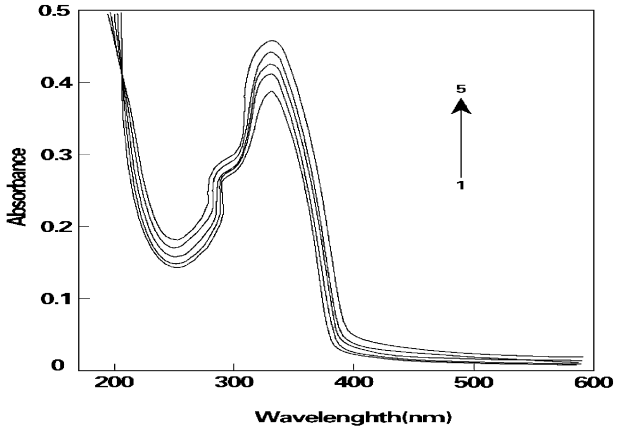


Figure 1. Absorption spectra of tranilast with various concentrations of ethylenediamine linked β-CD dimer from 1 to 5:0, 5.00×10^{-5}, 1.00×10^{-4}, 2.00×10^{-4}, and 3.00×10^{-4} mol L^{-1} of β-CD dimer, $C_{tranilast}$ = 2.00 μg mL^{-1}, pH 2.00.

Based on the inclusion reaction, tranilast in aqueous solution was spectrofluorimetrically determined with high sensitivity and selectivity. The linear range was $10.8–1.40 \times 10^4$ ng $mL^{-1}$with the detection limit 3.2 ng mL^{-1}. To our best knowledge, usage of β-CD dimer as the sensitizing agent for determination of tranilast in the aqueous solution by spectrofluorimetry has not been reported. The proposed method had been applied to the determination of tranilast in serum with satisfactory results.

2. Results and discussion

2.1. Excitation and emission spectra

The absorption spectra of tranilast were scanned for selecting an excitation wavelength for fluorescence measurements (Fig. 1). Figure 1 displays absorption spectra of tranilast at different concentrations of ethylenediamine linked β-CD dimer. As the β-CD dimer concentration increased, the absorption maximum of tranilast at 342 nm slightly redshifted 3 nm, and with a concomitant increase in the absorption intensity. There was an isosbestic point in the absorption spectra (Fig. 1), which indicated the formation of inclusion complex between β-CD dimer and tranilast.

Based on the absorption spectra (Fig. 1), the maximum absorption wavelength was chosen as maximum excitation wavelength for fluorescence. The excitation and emission spectra were scanned (Fig. 2). The maximum excitation and emission wavelengths were 345 and 460 nm, respectively, and a significant increase of the fluorescence intensity was observed when ethylenediamine linked β-CD dimer was added to the aqueous solution of tranilast.

2.2. Influence of pH

Because of the instability of cyclodextrin at very low pH, the use of strongly acidic solution containing β-CD was avoided.[24] Thus, the pH dependence of the system was studied over the range of 1.40–6.00. The results were

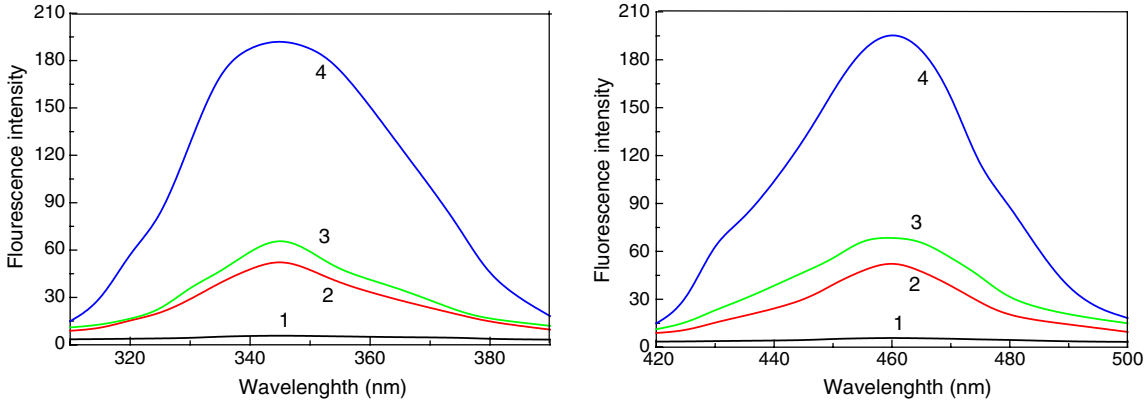


Figure 2. Excitation and emission spectra: (1) reagent blank; (2) tranilast solution; (3) tranilast + β-CD; (4) tranilast + β-CD dimer $C_{tranilast}$ = 2.00 μg mL^{-1}; pH 2.00; $C_{β\text{-}CD} = 4.00 \times 10^{-4}$ mol L^{-1}; $C_{β\text{-}CD\ dimer} = 2.00 \times 10^{-4}$ mol L^{-1}.

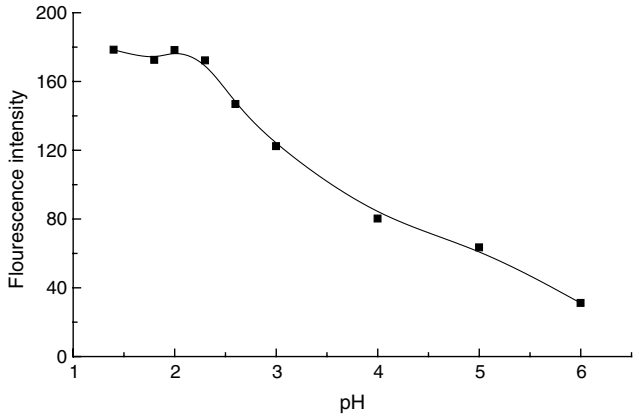


Figure 3. Influence of pH on the fluorescence intensity of the complex $C_{\text{tranilast}} = 2.00\ \mu g\ mL^{-1}$; $C_{\beta\text{-CD dimer}} = 2.00 \times 10^{-4}\ mol\ L^{-1}$.

shown in Figure 3. As can be seen, the fluorescence intensity was relatively high and almost remained constant over the pH range of 1.40–2.30. Therefore, a pH of 2.00 was fixed using KH_2PO_4–citric acid buffer solution.

As the volume of the buffer added (from 1.00 to 3.00 mL) had little effect on fluorescence intensity, 2.00 mL of buffer solution was used in subsequent experiments.

2.3. Influence of ethylenediamine linked β-CD dimer concentration

Influence of ethylenediamine linked β-CD dimer concentration on the fluorescence intensity of the complex was shown in Figure 4. As can be seen, with increasing concentration of ethylenediamine linked β-CD dimer the fluorescence intensity of the complex also increased. Thus, 3.00 mL of $1.00 \times 10^{-4}\ mol\ L^{-1}$ ethylenediamine linked β-CD dimer was used.

2.4. Influence of temperature and reaction time

The influence of temperature was tested. The experimental results showed that higher temperature increased the collision of molecules, which made the collision probability of excited molecules and solvent molecules increase and resulted in the declination of fluorescence intensity. So room temperature was selected.

The effect of reaction time was studied, the results (Fig. 5) showed that the fluorescence intensity reached a maximum after the reagents had been added for about 15 min and remained constant for at least 1 h. Hence, after inclusive reaction was carried out for 15 min, the subsequent fluorescence measurement was made at room temperature.

2.5. Apparent association constant

The apparent association constant of the inclusion complex can be determined by the following method: assuming that the composition of the complex is 1:1, it can be written as:

$$S + \beta\text{-CD dimer} \leftrightarrow (\beta\text{-CD dimer}) - S$$

where S denotes tranilast. The formation constant of the complex (K) is given by:

$$K = [(\beta\text{-CD dimer}) - S]/[S][\beta\text{-CD dimer}]$$

[β-CD dimer], [S], and [(β-CD dimer) − S] are equilibrium concentrations. An apparent association constant value for the inclusion complex can be determined through the typical double reciprocal plots: [25,26]

$$1/(F - F_0) = 1/[(F_\infty - F_0)KC_{(\beta\text{-CD dimer})}] + 1/(F_\infty - F_0) \quad (1)$$

where F is the observed fluorescence intensity of the tranilast solution at each ethylenediamine linked β-CD dimer concentration tested, F_0 and F_∞ are the fluorescence intensities in the absence of ethylenediamine linked β-CD dimer and when all the tranilast molecules are complexed, respectively. It is taken into account that: (1) ethylenediamine linked β-CD dimer is in a large excess with respect to tranilast and therefore its free and analytical concentrations are the same; (2) the variations in the fluorescence intensity are proportional to the complex concentrations and (3) at high ethylenediamine linked β-CD dimer concentration essentially all of the tranilast molecules are complexed.

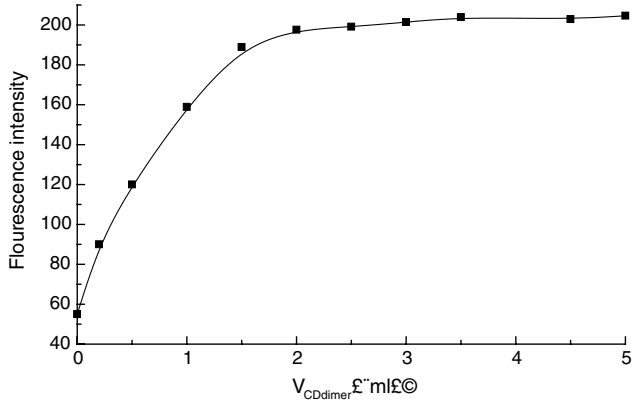


Figure 4. Influence of ethylenediamine linked β-CD dimer on the fluorescence intensity of the complex $C_{\text{tranilast}} = 2.00\ \mu g\ mL^{-1}$; pH 2.00; $C_{\beta\text{-CD dimer}} = 1.00 \times 10^{-4}\ mol\ L^{-1}$.

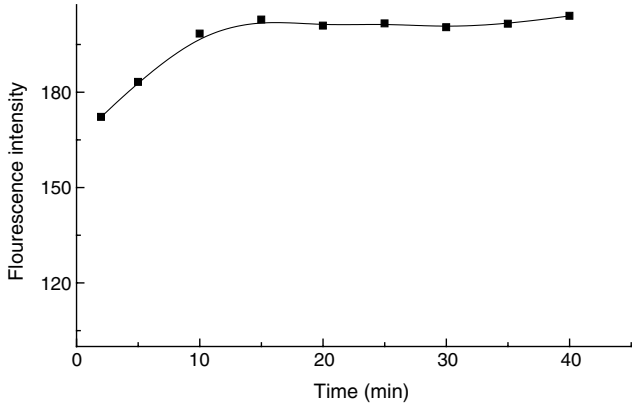


Figure 5. Effect of reaction time on fluorescence intensity of the complex $C_{\text{tranilast}} = 2.00\ \mu g\ mL^{-1}$; pH 2.00; $C_{\beta\text{-CD dimer}} = 3.00 \times 10^{-4}\ mol\ L^{-1}$.

The good linear relationship obtained when $1/(F-F_0)$ was plotted against $1/C_{(\beta\text{-CD dimer})}$ supported the existence of a 1:1 complex ($R = 0.9992$, Fig. 6). Its apparent association constant was determined to be 8.39×10^3 L mol^{-1}.

Assuming that tranilast and β-CD dimer form a 1:2 complex, it can be written as:

$$S + 2(\beta\text{-CD dimer}) \leftrightarrow (\beta\text{-CD dimer})_2 - S$$

where S denotes tranilast. The formation constant of the complex (K') is given by:

$$K' = [(\beta\text{-CD dimer})_2 - S]/[(\beta\text{-CD dimer})]^2[S]$$

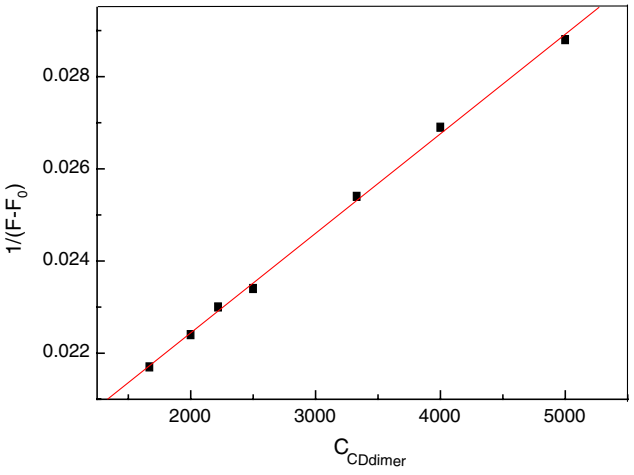


Figure 6. Plot of $1/(F-F_0)$ versus $1/C_{(\beta\text{-CD dimer})}$.

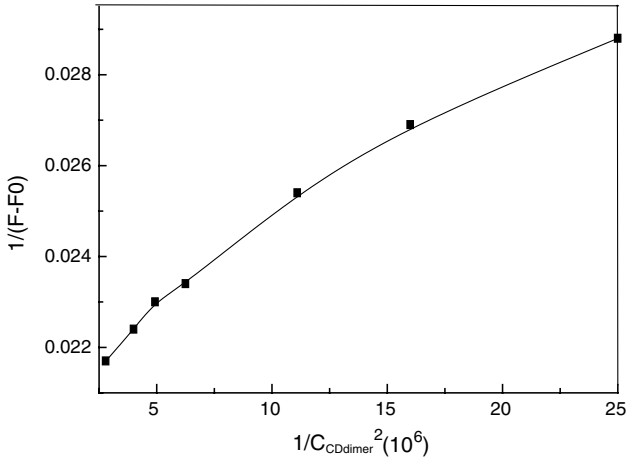


Figure 7. Plot of $1/(F-F_0)$ versus $1/C^2_{\beta\text{-CD dimer}}$.

If [β-CD dimer] ≫ [(β-CD dimer)$_2$ − S] ≫ [(β-CD dimer) − S], then the following expression is obtained:

$$1/(F-F_0) = 1/[(F_\infty - F_0)K'C^2_{\beta\text{-CD dimer}}] + 1/(F_\infty - F_0),$$

when making a plot of $1/(F-F_0)$ against 1/[β–CD dimer]^2, no linear relationship can be observed (Fig. 7), which indicated that the composition of the complex was not 1:2.

As a conclusion, the composition of the complex is 1:1 ((β-CD dimer): tranilast) with apparent association constant of 8.39×10^3 L mol^{-1}.

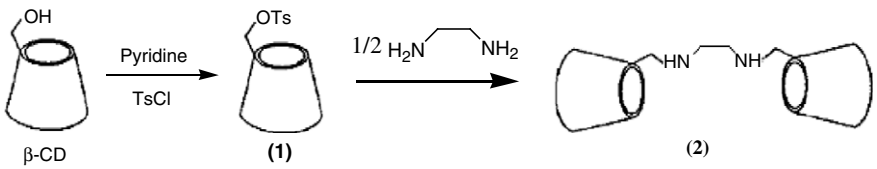
Based on the experimental results, it was interesting to note that the CD dimer connected with a linker of ethylenediamine, which possesses dual hydrophobic cavities in close proximity, can bind tranilast molecules to form supramolecular sandwich complexes[27] and thus give much higher binding abilities and molecular selectivities than those exhibited by parent CD. The possible process of the supramolecular interaction between β-CD dimer and tranilast (guest) was deduced as follows (Scheme 3).

2.6. Analytical characteristics

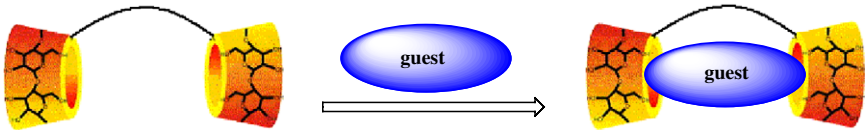
Under the optimum experimental conditions, there was a linear relationship between the fluorescence intensity and the concentration of tranilast in the range of 10.8–1.40×10^4 ng mL^{-1} with a correlation coefficient of 0.9991. The regression equation was $\Delta F = 33.88 + 123.77$[tranilast] (μg mL^{-1}). The detection limit, as defined by IUPAC,[28] was determined to be 3.2 ng mL^{-1} according to the formula of $C = KS_0/S$, where the value of K was taken as 3, the standard deviation was 0.134 obtained from a series of 11 reagent blanks, and S was the slope of the standard curve. The relative standard deviation (RSD) was 2.3% obtained from a series of 11 standards each containing 2.00 μg mL^{-1} of tranilast.

2.7. Influence of interference

The influence of the commonly used tablet excipients and the main constituents of serum on the determination of 2.00 μg mL^{-1} tranilast was studied. A 3000-fold mass excess of them over 2.00 μg mL^{-1} tranilast was tested first. If interference occurred, the ratio was reduced progressively until the interference ceased. The criterion for interference was fixed at a ±5.0% variation of the average fluorescence intensity calculated for the established level of tranilast. The results were shown in Table 1 and it was obvious that the determination was



Scheme 2. Synthesis of ethylenediamine linked β-CD dimer (**2**).



Scheme 3. The possible process of the supramolecular interaction.

Table 1. Effect of interference (tolerance error ± 5.0%)

Tolerance ratio in mass (m/m)	Interference
3000	K^+, Na^+, Cl^-
2500	Ca^{2+}, Mg^{2+}, Zn^{2+}, glucose, sucrose
2000	PO_4^{3-}, polyethylene glycol, glycin
1000	Boracic acid, gelatin, casein
800	Methylcellulose, sodium carboxymethylcelluose, starch
600	Mannitol, sorbitol, gum acacia power, tryptophan
400	Fe^{3+}, lactose, sodium acetate

free from interference of usual excipients and constituents of serum.

3. Applications

3.1. Determination of tranilast in serum

The procedure of serum sample analysis was used according to Ref. 29: The standard solution containing 0.400 mg tranilast was added into 4.00 mL serum of adult human. Then the solution was divided into two parts. One was diverted into 100 mL volumetric flask and diluted to volume with doubly distilled water. The other was mixed with acetonitrile according to the volume ratio of 1:2 in order to remove protein. The mixture was centrifuged for 5 min. Then the centrifugate was transferred into a 100 mL volumetric flask and diluted to volume with doubly distilled water. The results are given in Table 2. As can be seen, there was no interference from the serum constituents.

4. Conclusion

Based on the enhancement of the fluorescence intensity of tranilast, a spectrofluorimetric method for the determination of tranilast in bulk aqueous solution in the presence of β-CD dimer was developed. The supramolecular interaction of tranilast and ethylenediamine linked β-CD dimer has been studied by spectrofluorimetry. The results showed that β-CD dimer reacted with tranilast to form a 1:1 (host:guest) complex with an apparent association constant of 8.39×10^3 L mol^{-1}.

5. Experimental

5.1. Apparatus and reagents

All the spectrofluorimetric measurements were carried out on a Cary Eclipse (Varian, Australia) spectrofluorimeter equipped with a xenon lamp and 1.0 quartz cells. Absorption spectra were obtained from a UV-1700 (Shimadzu) UV–vis spectrophotometer. Infra-red spectra were obtained from a PE-983G IR-spectrophotometer (Perkin-Elmer). ^{1}H NMR and ^{13}C NMR spectra data were recorded on a Bruker Avance 300. Elemental analysis was performed on a Perkin Elmer SeriesIICHNS/O Analyzer. pH measurements were made with a pHS-3 digital pH-meter (Shanhai LeiCi Device Works, Shanhai, China) with a combined glass–calomel electrode.

Tranilast (99.8%) was used as received without further purification. Its stock solution (2.00 mg mL^{-1}) was prepared with ethanol. β-CD (purchased from China Medicine Group Shanghai Chemical Reagent Corporation) was purified by twice recrystallization in doubly distilled water, followed by vacuum drying at 80 °C. *p*-Tosyl chloride was purified by recrystallization in petroleum ether. Pyridine was dried over KOH (solid) for two days and then distilled prior to use. Other chemicals used were of analytical reagent grade. Doubly distilled water was used throughout.

5.2. Synthesis of ethylenediamine linked β-CD dimer

As shown in Scheme 2, 6-OTs-β-CD (**1**) was prepared by the reaction of *p*-tosyl chloride with β-CD in dry pyridine according to Refs. 30 and 31. Then, compound (**1**) was converted to ethylenediamine linked β-CD dimer (**2**) in 67.1% yield. Compound (**1**) (5.0 g) was dissolved

Table 2. Determination of tranilast in serum[a] ($n = 5$, $p = 95\%$)

Sample No.	Tranilast added (ng ml^{-1})	Tranilast found (ng ml^{-1})	Recovery (%)	RSD (%)
	50.0	51.7 ± 0.13	103	2.6
1	100	100 ± 0.09	100	1.5
	150	147 ± 0.17	98.0	1.8
	50.0	48.0 ± 0.12	96.0	2.4
2	100	99.6 ± 0.07	99.6	0.9
	150	151 ± 0.21	101	2.2

Sample 1 serum wasn't deproteinized with acetonitrile; Sample 2 serum was deproteinized with acetonitrile.
[a] The serum matrix signal was eliminated.

in 50 mL dry dimethylformamide (DMF), and 0.2 mL ethylenediamine was added into the mixture. The color of solution was yellowy. The mixture was reacted for 8 h at ~75 °C under argon. The reaction mixture was concentrated under reduced pressure and then poured into acetone (400 mL). The precipitates formed were collected on a glass filter and dried under reduced pressure. Compound (**2**) (0.97 g) was obtained after repeated recrystallization from water. IR (KBr, ν/cm^{-1}): 3381, 2928, 1638, 1403, 1334, 1246, 1156, 1079, 1030, 934, 839, 756, 706, 579, 531. ^1H NMR (300 MHz, DMSO-d_6, TMS): δ 5.6–5.7 (m, 26H, CD), δ 4.0–4.8 (m, 28H, CD), δ 3.3–3.6 (m, 84H, CD), δ 2.0–3.6 (m, 6H,C_6–$NHCH_2$–). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): δ 102.4 (1-C), δ 82.0 (4-C), δ 72.4–73.5 (2-C, 3-C, 5-C), δ 60.3 (6-C), δ 40.8 (C–NHR). Elemental analysis calcd (%) for $C_{86}H_{144}O_{68}N_2$: C, 45.03; H, 6.28; N, 1.22; O, 47.47; found: C, 45.21; H, 6.19; N, 1.11; O, 47.49. The decomposing temperature of the compound (**2**) was 202–204 °C.

5.3. Experimental procedure

5.3.1. Calibration graph. Into a series of 10 mL colorimetric tubes were added different aliquots of the tranilast stock solution containing 0–1.40×10^4 ng mL^{-1} of tranilast, 3.00 mL of 1.00×10^{-3} mol L^{-1} ethylenediamine linked β-CD dimer, and 2.00 mL of 0.20 mol L^{-1} KH_2PO_4–citric acid buffer solution (pH 2.00) sequentially. The mixture was diluted to mark with doubly distilled water, shaken thoroughly, and equilibrated at room temperature for 15 min. Then the fluorescence intensity of the solution was measured at 345/460 nm against a reagent blank.

5.3.2. Determination of the apparent association constant. Into a series of 10 mL colorimetric tubes were added 2.00 mL of 2.00 μg mL^{-1} tranilast, varied amounts of 1.00×10^{-3} mol L^{-1} ethylenediamine linked β-CD dimer, and 2.00 mL of 0.20 mol L^{-1} KH_2PO_4–citric acid buffer solution (pH 2.00) sequentially. The mixed solution was diluted to 10 mL with doubly distilled water, shaken thoroughly, and equilibrated at room temperature for 15 min. Then the fluorescence intensity of the solution was measured at 345/460 nm against a reagent blank.

Acknowledgments

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