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ORIGINAL ARTICLE

The synthesis and molecular recognition study of β-cyclodextrin derivatives modified by spiro[2H-benzopyran-2,2′-indoline]

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Abstract In this study, we adopted the condensation reaction between the aldehyde group in spiro[2H-benzopyran-2,2′-indoline] and the ethylene diamine in β-cyclodextrins to synthesize 1,3,3-trimethyl-6′-mono-(6-deoxy-ethylenediimine-β-cyclodextrin)-spiro[2H-benzopyran-2,2′-indoline] (β-CD-SPBI). The structure of the target compound was characterized by elemental analysis, NMR and IR spectroscopy. The UV–Vis and fluorescence spectroscopy were investigated in different solvents. The preliminary study of its molecular recognition was also conducted. The results indicated that β-CD-SPBI existed as two structural isomers, both of which showed significant fluorescent characteristics and β-CD-SPBI could showed the capacity of dual molecular recognition of a drug, ribavirin.

Keywords Spiro[2H-benzopyran-2,2′-indoline] · Cyclodextrin derivative · Spectral properties · Molecular recognition

Abbreviations

β-CD	β-Cyclodextrin
β-CD-SPBI	1,3,3-Trimethyl-6′-mono-(6-deoxy-ethylenediimine-β-cyclodextrin)-spiro[2H-benzopyran-2,2′-indoline]

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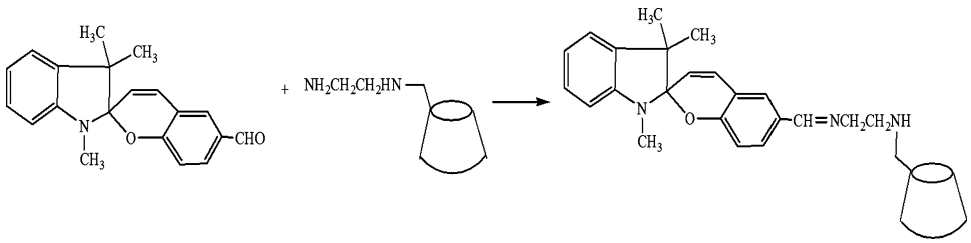
Introduction

In recent years, with the development in cyclodextrin supermolecular chemistry, studies involving cyclodextrin multifunctional modification attracted great attentions [1–3]. Through the modification of photochromic molecules, we could enhance their molecular recognition capability for guest molecules, and thus expand their applications in various fields. Researches in the area of molecular detection and recognition have been progressed further by utilization of changes in the fluorescence intensity of cyclodextrin derivatives [4–6]. Spiro[2H-benzopyran-2,2′-indoline] derivatives are photochromic compounds and they have been widely used in optical information storage technology, optical recording material and other fields. Recently studies have shown that spiropyran exhibited high sensitivity and selectivity for metal ions detection by using their photochromic properties and fluorescence [7]. By introducing crown ether building block into spiropyran structure and combining their interactions with metal ions, it has proved that metal ions can induce structural isomerization of spiropyran and thus spiropyrans are superior candidates in photochemical control and spectrophotometric determination of metal ions [8]. Shao et al. [9] coupled spiropyran and porphyrin to yield a highly selective fluorescence sensor for copper (II) cations. So far, however, there have been little reports on introducing cyclodextrin building blocks to spiropyrans as new supramolecules [10]. This study used an aldehyde functionalized spiro[2H-benzopyran-2,2′-indoline] and ethylene diamine-cyclodextrin as starting materials, through aldehyde–amine condensation reaction to synthesize cyclodextrin derivatives with spiro[2H-benzopyran-2,2′-indoline] (Scheme 1). We confirmed the proposed structure of the product and studied its UV–Vis,

Scheme 1 Synthesis route to cyclodextrin modified by a spirobenzopyran



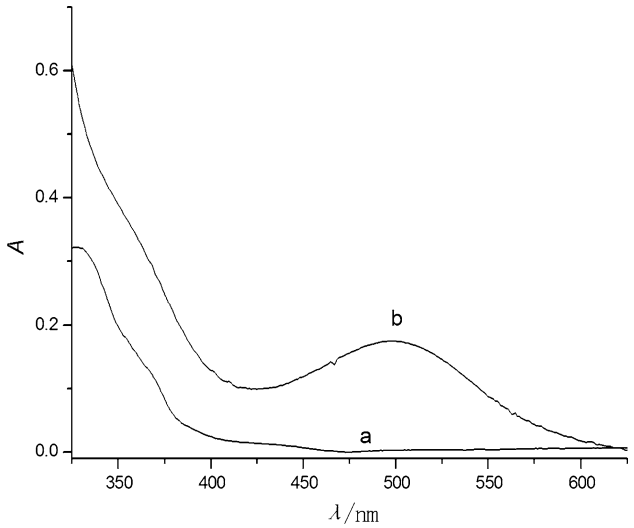


Fig. 1 UV–Vis absorption spectra of compound β-CD-SPBI in DMF (*a*) and aqueous solution (*b*)

fluorescence spectra. Based on the preliminary study of the molecular recognition of the model drug, ribavirin, (1-(β-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide), we anticipate that the product can be used for biomolecular recognition as well as drug analyses and detection.

Experiment section

Materials and instrument analyses

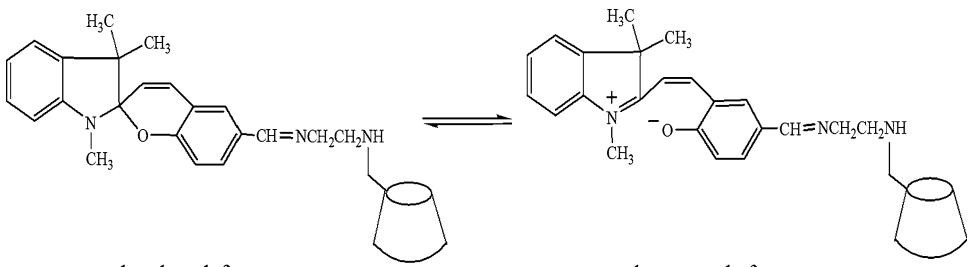
Beta-CD was obtained from Shan-Tou Chemical Factory in China, and purified two times by recrystallization from water before using. Other reagents were all analytical-grade made in China, and were used as received without further purification.

^{1}H-NMR measurements were conducted on a Varian Inova 400 spectrometer (Massachusetts) at room temperature with DMSO-d_6 as a solvent. 2D-NMR experiments were recorded on a Avance 500 spectrometer (Bruker, Switzerland) operating in DMF-d_7 at 298K. FT-IR spectroscopy experiments were preformed on a IR Prestige-21 model (Shimadzu, Japan) using the KBr method. Fluorescence spectrophotometer were used by RF-5301PC (Shimadzu, Japan). Elemental analyses were carried out on a Vario EL III instrument (Hanau, Germany). Ultraviolet–visible spectra were recorded on a SPECORD-50 model (Analytik Jena AG, Germany).

Experimental

To a round bottom flask, 0.16 g (0.52 mmol) of 1,3,3-trimethyl-6′-formyl-spiral(2H-benzopyran-2,2′-indoline) (SPBI) [11] was added. DMF (1 mL) was added to yield a purple solution upon dissolving the substrate. A 1.5 mL DMF yellow solution of 0.32 g (0.27 mmol) of 6-deoxy-6-(2-aminoethyl)-amino-β-cyclodextrin (β-CD-EDA) [12] was added to the above flask and the mixture was stirred at 30 °C for 8 h. The reaction was stopped by addition of acetone to precipitate the brown crude product. The yellow–brown colored pure product was obtained by silica gel column chromatography with a yield of 25.5%. ^{1}H NMR (DMSO-d_6), δ: 1.10 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 2.66–2.89 (m, 8H, N–CH_3 CH_2CH_2NH), 3.36–3.63 (m, 42H, β-CDC_2, C_3, C_4, C_5, C_6H), 4.49 (s, 6H, O_6H), 4.83 (s, 7H, C_1H), 5.73 (b, 15H, O_2H, O_3H, –CH=C*H*–), 6.57–6.59 (d, 1H –C*H*=CH–), 6.72–7.95 (m, 7H, ArH), 8.23 (s, 1H, –C*H*=N–); FT-IR, ν/cm^{-1}, 3428, 1647, 1609, 1487, 1035;

Scheme 2 Two isomers of β-CD-SPBI



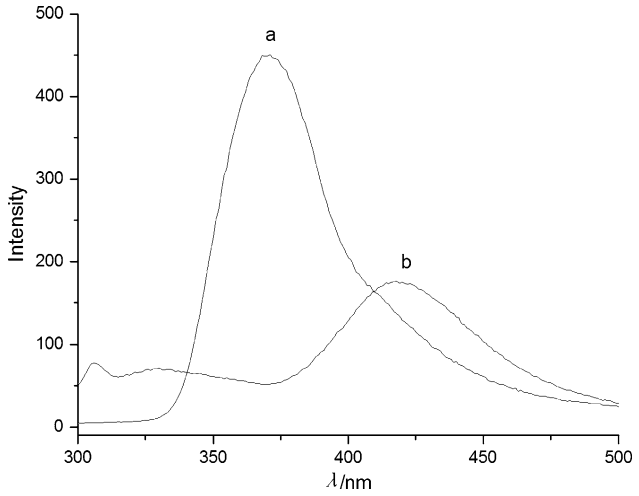


Fig. 2 Fluorescence spectra of compounds in DMF. *a* β-CD-SPBI λex/λem = 280/369 nm $c_{\beta\text{-CD-SPBI}} = 1.99 \times 10^{-4}$ mol L^{-1}. *b* SPBI λex/λem = 280/419 nm $c_{\text{SPBI}} = 5.41 \times 10^{-5}$ mol L^{-1}

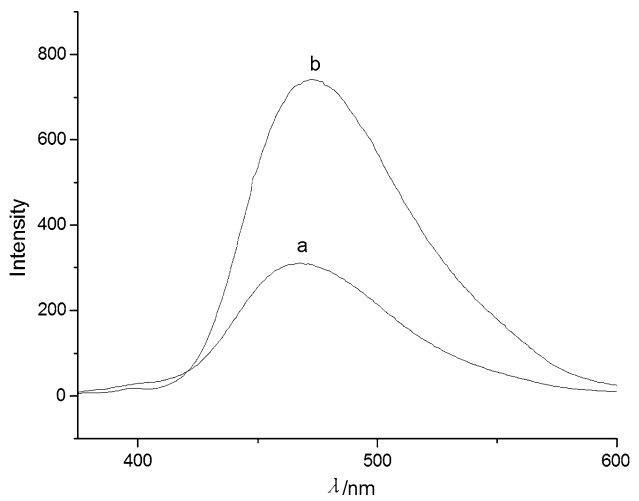


Fig. 3 Fluorescence spectra of compounds in aqueous solution. *a* β-CD-SPBI λex/λem = 350/467 nm $c_{\beta\text{-CD-SPBI}} = 2.13 \times 10^{-4}$ mol L^{-1}. *b* SPBI λex/λem = 350/472 nm $c_{\text{SPBI}} = 3.47 \times 10^{-5}$ mol L^{-1}

the composition of β-CD-SPBI was found to be $C_{64}H_{93}O_{35}N_3{\cdot}5H_2O$ (Calcd. C 49.45, H 6.63, N 2.70; found: C 49.87, H 6.35, N 2.29).

Results and discussion

β-CD-SPBI spectroscopic properties

Figure 1 is the UV–Vis absorption spectrum of β-CD-SPBI in DMF and deionized water. From its UV–Vis absorption, it is clear that β-CD-SPBI has no absorption within the visible region in DMF, whereas it absorbs with a maximum at 498 nm in water, indicating that this compound exists as

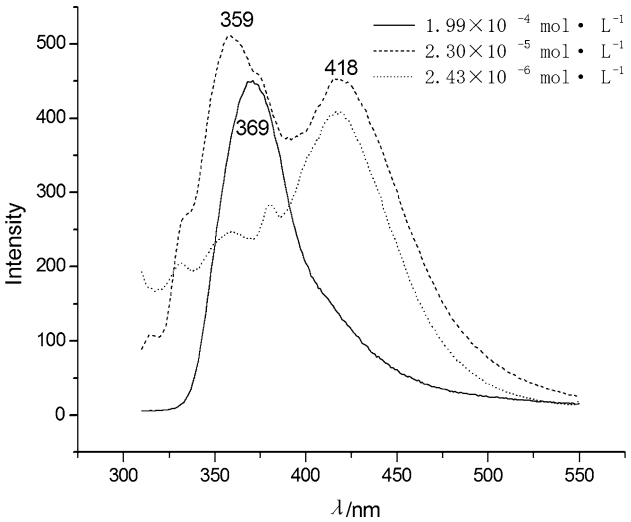


Fig. 4 Fluorescence spectra of β-CD-SPBI in DMF at various concentrations

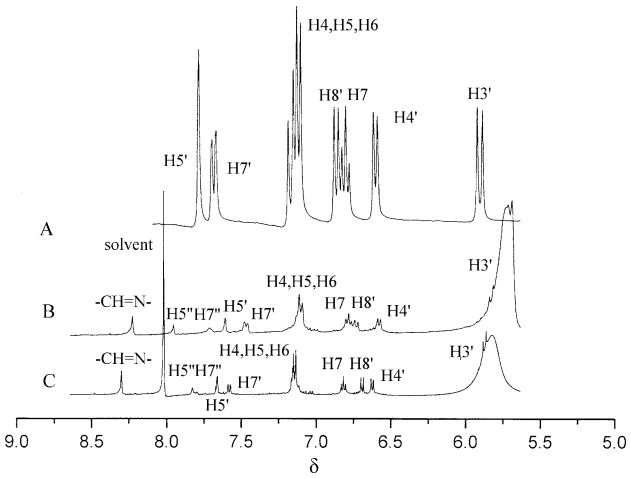


Fig. 5 ^{1}H NMR spectra of SPBI in DMSO (*A*), β-CD-SPBI in DMSO (*B*) and β-CD-SPBI in DMF (*C*)

the close form of spiropyran and the open form of merocyanine in DMF and water, respectively (Scheme 2).

To investigate the fluorescence response of β-CD-SPBI, we measured the fluorescence spectra of β-CD-SPBI and SPBI in DMF and deionized water. Due to the existence of two different structures of β-CD-SPBI in DMF and water, i.e., the close form spiropyran and the open form merocyanine, β-CD-SPBI has the different fluorescence spectra. As can be seen in Figs. 2 and 3, the maximal emission peak is 369 nm when β-CD-SPBI was excited at 280 nm in a DMF solution; however, the maximal emission wavelength is 467 nm when β-CD-SPBI was excited at 369 nm in an aqueous solution. Moreover, Fig. 3a, b shows the fluorescence spectra of β-CD-SPBI and SPBI under the same excitation

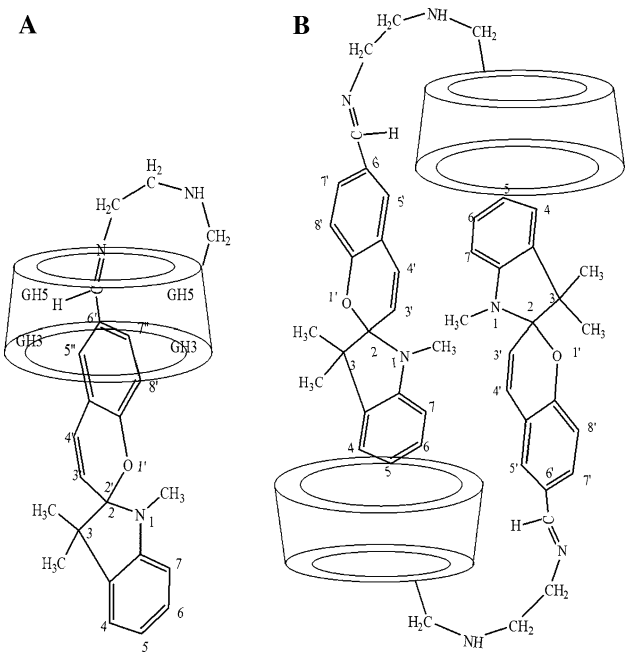


Fig. 6 Possible conformation of β-CD-SPBI in DMF

wavelength (350 nm) in water. The β-CD-SPBI and SPBI have the similar maximal emission wavelength at 467 and 472 nm, respectively. This emission similarity herein can be explained by the following. In water, both β-CD-SPBI and SPBI exist predominantly in the open merocyanine form, due to the increase of conjugated chain length, and the fluorescence emission shows a red shift.

Based on the fluorescence spectra of β-CD-SPBI and SPBI (Fig. 2a, b), spiropyran (SPBI) after cyclodextrin modification significantly changed its fluorescence behavior. Under the same excitation condition (the same wavelength), its maximal emission wavelength showed a blue shift with an enhanced fluorescence intensity. Figure 4 shows fluorescence emission spectra for β-CD-SPBI with different concentrations DMF solvent. The maximum fluorescence emission wavelength of 369 nm at the solution concentration of β-CD-SPBI is about 1.99×10^{-4} mol L^{-1}. The solution concentration in very dilute at about 2.43×10^{-6} mol L^{-1}, its maximum emission wavelength red moved to 418 nm. And when the solution concentration is at about 2.30×10^{-5} mol L^{-1}, its maximum emission wavelength peaks are 359 and 418 nm, respectively. This observation indicates that different concentrations of β-CD-SPBI solution, there are different β-CD-SPBI the molecular aggregation state, the high concentration demonstrates that inter-molecular association provides more compact aggregates with inclusion interaction. While β-CD-SPBI solution is in dilute, spiropyran units out of the cyclodextrin cavity with a maximum emission wavelength of 418 nm is similar to the monomeric spiropyran.

To confirm this speculation and in-depth analysis of the "self-organization" behavior of β-CD-SPBI, its conformations in DMF were studied by 2D-NMR spectroscopy. If the β-CD motif functions as a simple substituent with no significant effect on the conformational changes of β-CD-SPBI, the ^{1}H NMR spectrum of SPBI domain in β-CD-SPBI should be similar to the parent SPBI as shown in Fig. 5A. The only possible changes should appear at the chemical shift of H5′ and H7′ on the aromatic ring, due to the imine condensation of the C6′-aldehyde. However, not only the chemical shifts of H5′ and H7′ change, the absorption peaks of H5′ and H7′ change from the original two sets to four sets [7.98(s), 7.74(d), 7.63(s), 7.50(d)] (Fig. 5B in DMSO) of similar splitting patterns to the parent SPBI [H5′ 7.78(s); H7′, 7.67(d)]. This can be inferred that the benzopyran rings baring H5′ and H7′ are in two distinct chemical environments: inside and outside the cavities of β-CDs. The NOESY spectrum of β-CD-SPBI (Fig. 7B) further confirms this speculation. The observed correlations of H5′, H7′ (shown as H5″ and H7″ in Fig. 7B) with H3, H5 in the cavity β-CD indicates that the phenyl region of the benzopyran unit is in the cavity of β-CD. We would like to point out that in the ^{1}H NMR spectrum of β-CD-SPBI in DMF (Fig. 5C), the signals of H5″ and H7″ were observed as apparent two adjacent singlets. This may imply the different orientation of aromatic ring enclosed in the β-CD cavity caused by different solvents [13]. Furthermore, the correlation of H3, H5 in the β-CD cavity with the proton in the imine group that tethers SPBI and β-CD (Fig. 7A, B, unlabeled are solvent peaks) indicates a "shelf-inclusion" form of β-CD-SPBI (Fig. 6A). Given the size of SPBI, this "shelf-inclusion" form of β-CD-SPBI should generate in the imine condensation process involves partial inclusion mechanism. Based on the integration ratio of the H5′/H7′ and H5″/H7″ peaks, 30% of β-CD-SPBI are the "shelf-inclusion" conformers, and the other 70% are the "self-organized" dimeric conformers as shown in Fig. 6B. The NEOSY correlation between H2/H4 and imine proton/H7′; H4/H5/H6 in the indoline ring and H4′/H5′/2,3-OH in β-CD confirms the existence of the dimeric conformer, which is resulted by the synergistic effect of π-π stacking, hydrophobic interaction and Van der Waals forces.

Based on the conformational studies on β-CD-SPBI in DMF, we believe that the dimeric conformer dominants at high concentration of β-CD-SPBI. At this stage, the ring opening of SPBI is suppressed. Fluorescent emission of shorter wavelength is obtained upon UV-lights excitation. At low concentration, the monomeric form of β-CD-SPBI dominants. The spiropyran is prone to open under UV-lights excitation and lead to a red-shifted fluorescence.

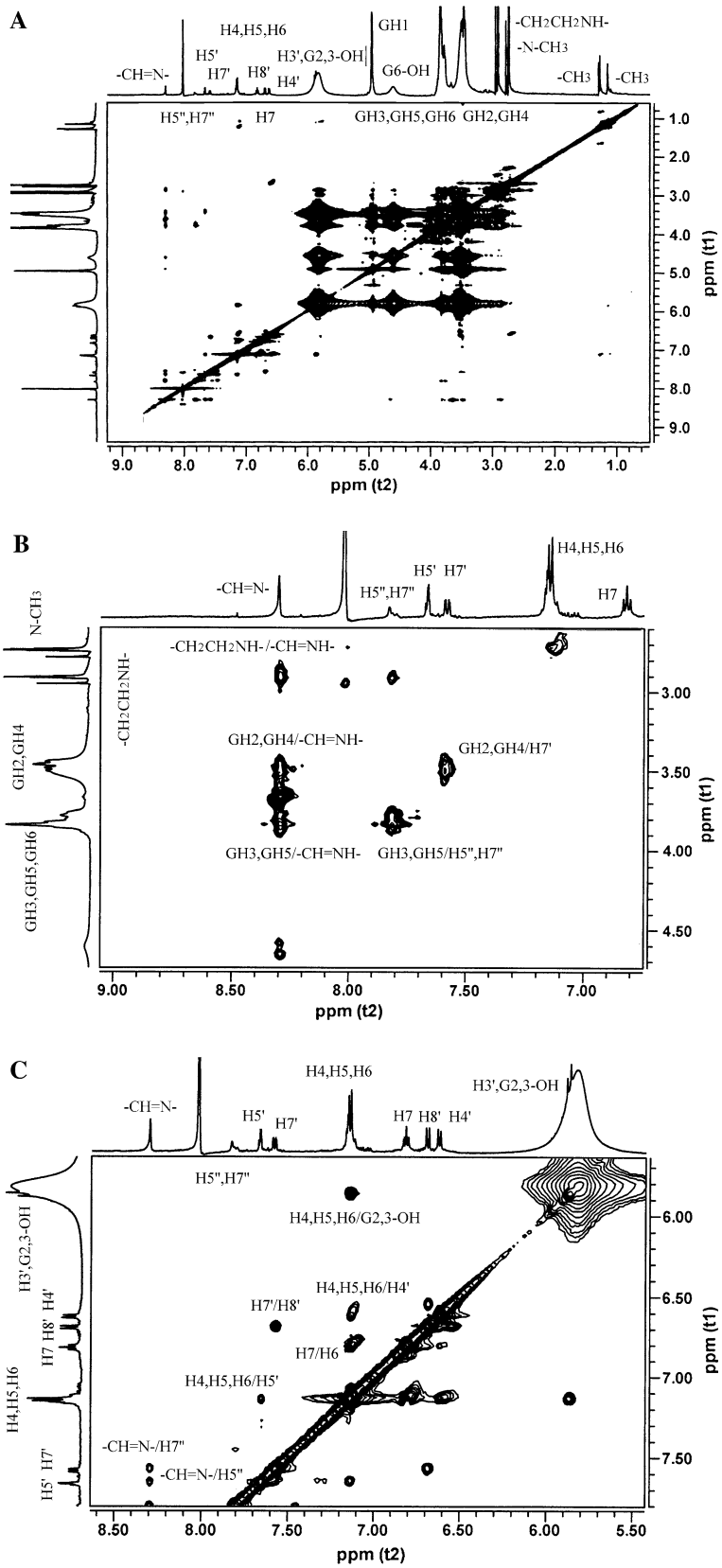


Fig. 7 2D NOESY spectrum of β-CD-SPBI (2.13×10^{-2} mol L^{-1}) in DMF at 298 K

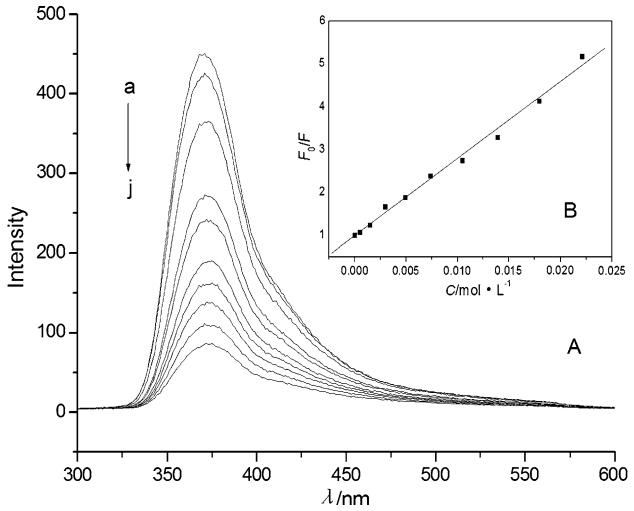


Fig. 8 **A** Fluorescence spectra of β-CD-SPBI in DMF at various ribavirin concentrations $c_{\beta\text{-CD-SPBI}} = 1.99 \times 10^{-4}$ mol L^{-1} λex = 280 nm. The ribavirin concentrations a–j are from 0 to 2.21×10^{-2} mol L^{-1}. **B** The Stern–Volmer plots of fluorescence quenching of β-CD-SPBI in DMF at various ribavirin concentrations

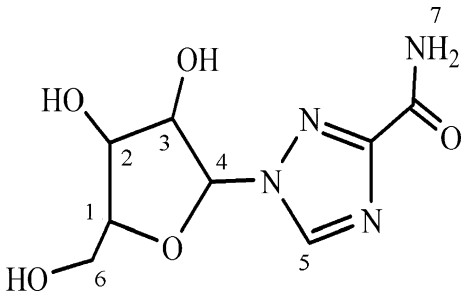


Fig. 9 Molecular structure of the ribavirin

β-CD-SPBI molecular recognition performance

The β-CD modified fluorescent probe is expected to exhibits better molecular recognition ability. The molecular recognition ability of β-CD-SPBI is assayed by the fluorescent quenching by ribavirin with β-CD-SPBI in various concentration in DMF. Figure 8 shows the fluorescence spectra of β-CD-SPBI in higher concentration (1.99×10^{-4} mol L^{-1}) with addition of ribavirin (Fig. 9) in DMF. We can clearly observe that the fluorescence intensity of β-CD-SPBI gradually decreased with the addition of ribavirin in DMF. According to experimental data in Fig. 8, the Stern–Volmer curve (Fig. 8B, $F_0/F = 0.9855 + 179.80C$, $r = 0.9961$) was obtained by calculating the correlation of fluorescence intensity of β-CD-SPBI (F_0/F) and the concentration of ribavirin. With the increase of ribavirin concentration, F_0/F gradually increases and they show a good linear relationship. The slope is the rate constant of fluorescence quenching with a value of $K = 179.80$ L mol^{-1}.

To investigate the mechanism of the interaction between ribavirin and β-CD-SPBI, we examined the NOESY spectra of β-CD-SPBI in DMF in the presence of ribavirin (Fig. 10). The correlations of H7 in ribavirin amide with H5/H6 in the β-CD cavity; H4/H5 in ribavirin with 2,3-OH of β-CD and H2/H4 outside the β-CD cavity indicate the strong interaction between ribavirin and β-CD. On the other hand, the attenuated characteristic correlation signals of the dimeric conformer of β-CD-SPBI (H4/H5/H6 in the indoline ring with H4′/H5′ in the pyran ring and 2,3-OH in β-CD) reveal the disaggregation of the β-CD-SPBI dimer promoted by ribavirin, due to the strong hydrogen bonding between ribavirin and β-CD. Therefore, the fluorescent β-CD-SPBI intensity also decreases accordingly.

Figures 11 and 12 shows the fluorescent spectra of 10^{-5} and 10^{-6} mol L^{-1} of β-CD-SPBI in DMF in the presence of ribavirin. A certain rule is observed that the fluorescence intensity of β-CD-SPBI at UV region decreases, but the fluorescence intensity at visible region increases when the concentration of β-CD-SPBI decreases and the concentration of ribavirin increases. We can rationalize this observation by the disaggregation of β-CD-SPBI upon dilution and the enrichment of ribavirin, which lead to the decrease of fluorescence intensity at UV region. At the same time, the open merocyanine form of SPBI increases lead to the increase of fluorescence intensity at visible region.

As can be seen in Fig. 13, and unlike in the DMF solution, in aqueous solution the fluorescence intensity of β-CD-SPBI increases gradually with the addition of ribavirin, which indicates that the interaction mechanisms of ribavirin and β-CD-SPBI are different in different solvents. In aqueous solutions, the spiropyran moiety is solvated in the open form of merocyanine. Because ribavirin can form complexes with the N, O-heteroatoms with the open form merocyanine through hydrogen bonding, it is beneficial to stabilize the open form of spiropyran as the merocyanine and we believe this is the reason for the enhancement of fluorescence intensity. The enhancement not only comes from the concentration increase of the open form of spiropyran, but also comes from the complex by ribavirin and the open form of spiropyran, which effectively retards the nonradioactive deactivation of the chromophores by light induced structural tautomerization and a similar process has been reported in literature [9] about the complexes from the spiropyran derivatives and metal ions. Therefore, the distinct differences of fluorescence intensity result from different compounds interacting with β-CD-SPBI can be used as the basis for molecular recognition.

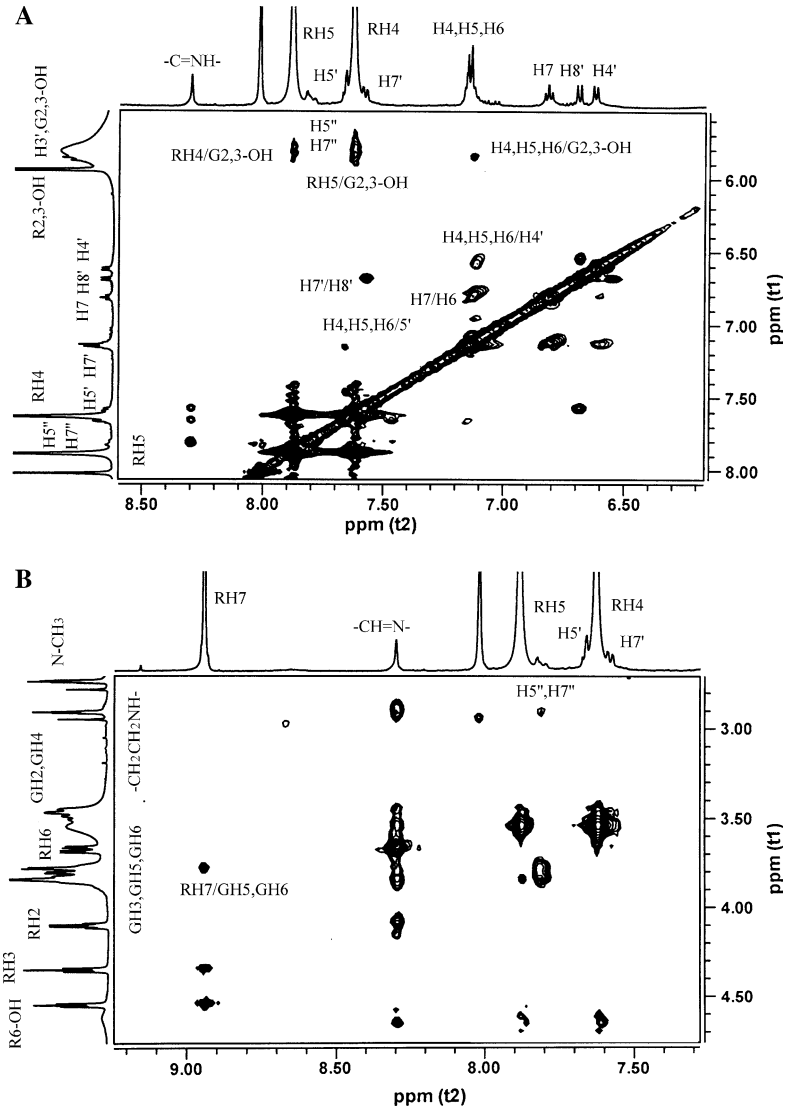


Fig. 10 2D NOESY spectrum of β-CD-SPBI (2.13×10^{-2} mol L^{-1}) and ribavirin (8.56×10^{-2} mol L^{-1}) in DMF at 298 K

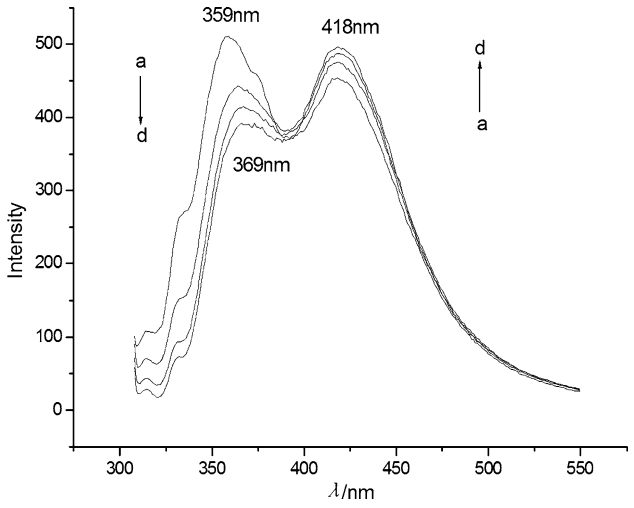


Fig. 11 Fluorescence spectra of β-CD-SPBI in DMF at various ribavirin concentrations $c_{\beta\text{-CD-SPBI}} = 2.30 \times 10^{-5}$ mol L^{-1} $\lambda ex =$ 300 nm. The ribavirin concentrations a–d are from 0 to 3.30×10^{-5} mol L^{-1}

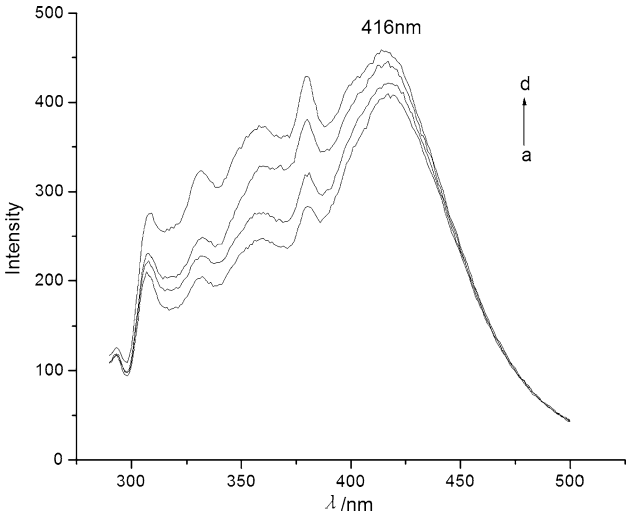


Fig. 12 Fluorescence spectra of β-CD-SPBI in DMF at various ribavirin concentrations $c_{\beta\text{-CD-SPBI}} = 2.43 \times 10^{-6}$ mol L^{-1} $\lambda ex =$ 280 nm. The ribavirin concentrations a–d are from 0 to 3.80×10^{-5} mol L^{-1}

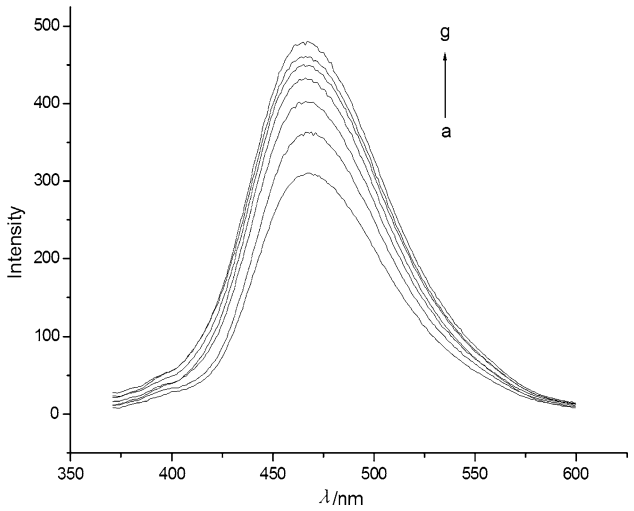


Fig. 13 Fluorescence spectra of β-CD-SPBI in aqueous solution at various ribavirin concentrations $\lambda ex/\lambda em = 350/467$ nm $c_{\beta\text{-CD-SPBI}} = 2.13 \times 10^{-4}$ mol L^{-1}. The ribavirin concentrations a–d are from 0 to 1.73×10^{-3} mol L^{-1}

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