# Molecular Recognition of Bridged Bis(β-cyclodextrin)s Linked by Phenylenediseleno Tether on the Primary or Secondary Side with Fluorescent Dyes†

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**A novel β-cyclodextrin dimer, 2,2′-*o*-phenylenediseleno-bridged bis(β-cyclodextrin) (2), has been synthesized by reaction of mono-[2-*O*-(*p*-tolylsulfonyl)]-β-cyclodextrin and poly(*o*-phenylenediselenide). The complexation stability constants ($K_s$) and Gibbs free energy changes ($-\Delta G°$) of dimer 2 with four fluorescence dyes, that is, ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS), Acridine Red (AR) and Rhodamine B (RhB) have been determined in aqueous phosphate buffer solution (pH = 7.2, 0.1 mol·L$^{-1}$) at 25 ℃ by means of fluorescence spectroscopy. Using the present results and the previously reported corresponding data of β-cyclodextrin (1) and 6,6′-*o*-phenylenediseleno-bridged bis(β-cyclodextrin) (3), binding ability and molecular selectivity are compared, indicating that the bis(β-cyclodextrin)s 2 and 3 possess much higher binding ability toward these dye molecules than parent β-cyclodextrin 1, but the complex stability constant for 2 linked from the primary side is larger than that of 3 linked from the secondary side, which is attributed to the more effective cooperative binding of two hydrophobic cavities of host 3 and the size/shape-fit relationship between host and guest. The binding constant ($K_s$) upon inclusion complexation of host 3 and AR is enhanced by factor of 27.3 as compared with that of 1. The 2D $^1$H NOESY spectrum of host 2 and RhB is performed to confirm the binding mode and explain the relative weak binding ability of 2.**

**Keywords** oganoselenium-bridged bis(β-cyclodextrin), molecular recognition, inclusion complexation, binding mode

## Introduction

As a kind of typical molecular receptors, cyclodextrin and its derivatives can bind a series of substrates (guests) to form host-guest complexes in aqueous solution.[1-3] Therefore, they can be extensively used in many fields of science and technology, serving as drug carriers,[4,5] artificial enzymes[6,7] and chemical sensors,[8,9] *etc*. To extend the binding ability of mono-modified cyclodextrins, the design and synthesis of novel bridged bis(cyclodextrin)s have been taken on to achieve the cooperative binding of the dual hydrophobic cavities to one guest molecule.[10-12]

In previous works,[13,14] we prepared a series of organoselenium-bridged bis(β-cyclodextrin)s and examined their molecular recognition behaviors with organic dyes. The results indicated that the bridged bis(β-cyclodextrin)s, possessing dual hydrophobic cavities in a close vicinity, significantly enhance the original molecular binding ability of the parent β-cyclodextrin through the cooperative binding of one guest molecule in the closely located two cyclodextrin cavities. Simultaneously, the comparison of complexation behavior of the 6,6′- and 2,2′-bridged bis(β-cyclodextrin)s linked by trimethylenediseleno tether indicated that the molecular binding ability of dimer tethered from the secondary side is stronger than that tethered from the primary side.

In present work, we have prepared a novel 2,2′-*o*-phenylenediseleno-bridged bis(β-cyclodextrin) (**2**) tethered by phenylenediseleno moiety and investigated the inclusion complexation behavior of **2** and 6,6′-*o*-phenylenediseleno-bridged bis(β-cyclodextrin) (**3**) with some organic dye guests (Chart 1) by using spectrofluoremetric titrations at 25 ℃ in aqueous phosphate buffer solution (pH = 7.2). It is of our special interesting to examine and compare the molecular binding ability of bridged bis(β-cyclodextrin)s linked from primary and secondary side, respectively. The result indicate that differing from the trimethylenediseleno-bridged bis(β-cyclodextrin)s reported previously by us,[14] 6,6′-bridged dimer **3** linked from the primary side by *o*-phenylenediseleno tether can form more stable complex with organic dyes than 2,2′-bridged dimer **2** linked from the secondary side.

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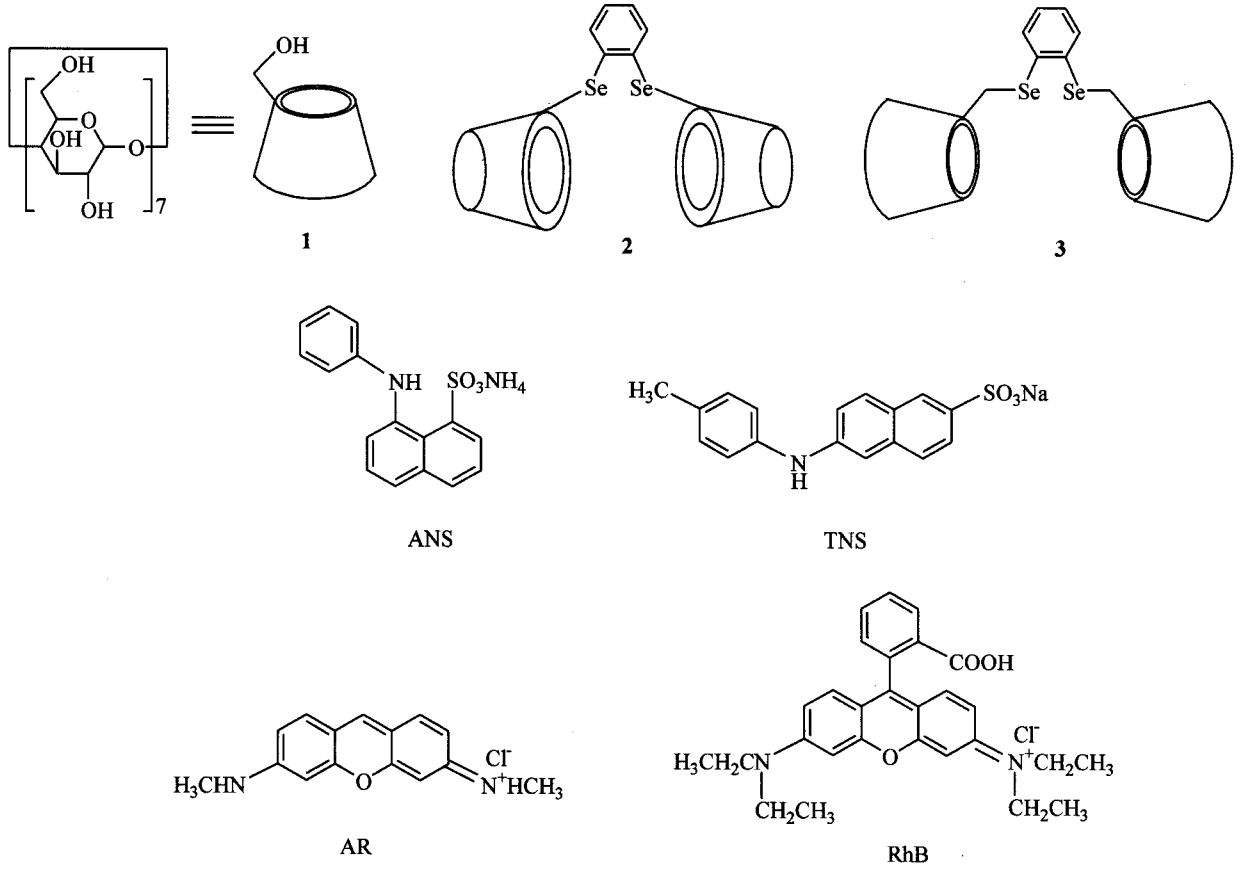
Received January 26, 2003; revised April 18, 2003; accepted May 5, 2003.
Project supported by the National Natural Science Foundation of China (Nos. 29992590-8 and 20272028), the Natural Science Fund of Tianjin (No. 013613511) and Special Fund for Doctoral Program from the Ministry of Education of China (No. 20010055001).
†Dedicated to Professor ZHOU Wei-Shan on the occasion of his 80th birthday.

**Chart 1**




## Experimental

*General procedure*

Elemental analyses were performed on a Perkin-Elmer 2400C instrument. $^1$H NMR spectra were recorded on a Varian INVOA300 instrument at 300 MHz in $D_2O$. FT-IR and UV spectra were recorded on a Nicolet FT-IR 5DX and Shimadzu UV-2401 spectrometer, respectively. Fluorescence spectra were measured in a conventional quartz cell (10 mm × 10 mm × 45 mm) at 25 ℃ on a JASCOFP-750 spectrometer equipped with a temperature controller and with excitation and emission slits of 5 nm width.

*Materials*

All guest dyes, *i. e.*, ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium 6-(*p*-toluidinyl)-2-naphthalenesulfonate (TNS), Acridine Red (AR), and Rhodamine B (RhB), were commercially available and used without further purification. β-Cyclodextrin of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried *in vacuo* at 95 ℃ for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 d and then distilled under a reduced pressure prior to use. All other chemicals were of reagent grade and were used without further purification. 6,6′-*o*-Phenymenediseleno-bridged bis(β-cyclodextrin) (**3**) were synthesized according to the reported procedures.[13] Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 mol·L$^{-1}$ phosphate buffer solution (pH = 7.2) for spectral titration.

*Synthesis*

2,2′-*o*-Phenylenediseleno bridged bis(β-cyclodextrin) (**2**) was prepared from mono-[2-*O*-(*p*-tolylsulfonyl)]-β-cyclodextrin (2-OTs-β-CD)[15] and poly (*o*-phenylenediselenide)[16] according to the following procedure. A solution of poly (*o*-phenylenediselenide) (0.117 g, 0.5 mmol), NaOH (0.06 g, 1.5 mmol) and $NaBH_4$ (0.06 g, 1.5 mmol) in dry ethanol (25 mL) was stirred under nitrogen at 85 ℃ for 15 min. When the color of the mixture disappeared, a solution of 2-OTs-β-CD (1.32 g, 1 mmol) in dry DMF (40 mL) was added dropwise into the clear solution over 1 h with magnetic stirring under $N_2$. The solution was heated to reflux for 36 h, and then the resultant mixture was evaporated under a reduced pressure, leaving a yellow solid. The residue was dissolved in water, and then acetone was added to the solution to give a yellow precipitate. The crude product was purified by column chromatography over Sephadex G-25 with distilled, deionized water to give a pure sample 130 mg, yield 10%. $^1$H NMR ($D_2O$, TMS) δ: 3.0—4.0 ($C_2$—$C_6$H, 84H), 4.7—4.9 ($C_1$H, 14H), 7.0—8.0 (ArH, 4H); $^{13}$C NMR (DMSO-$d_6$, TMS) δ: 127.4, 101.8, 81.4, 72.9, 72.3, 72.0,

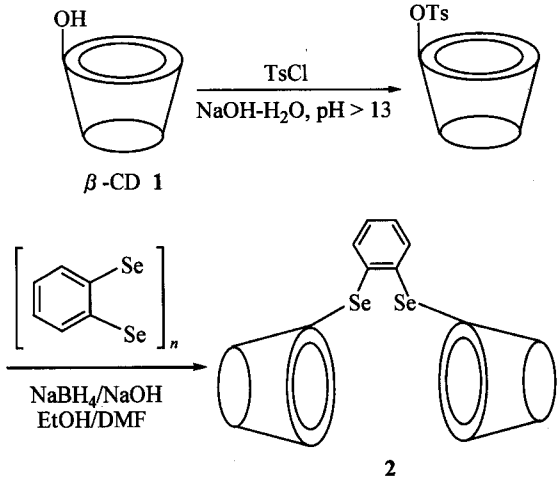
69.7, 59.8, 48.4; FT-IR (KBr) $\nu$: 3315, 2920, 1641, 1594, 1511, 1435, 1362, 1245, 1152, 1029, 931, 848, 755, 707, 579 $cm^{-1}$. Anal. calcd for $C_{90}H_{142}O_{68}Se_2 \cdot 10H_2O$: C 40.79, H 6.16; found C 40.60, H 6.15.

## Results and discussion

*Synthesis*

2,2′-*o*-Phenylenediseleno-bridged bis(β-cyclodextrin) (**2**) was prepared according to Scheme 1.

**Scheme 1**




*Spectral titrations*

In the titration experiments using fluorescence spectrometry, the fluorescence intensity of dyes (5—10 μmol·$L^{-1}$) gradually increased upon the addition of varying concentrations of hosts **2** and **3**, while the emission peak progressively shifts to the blue. The emission peak changes of the organic dyes with the addition of host cyclodextrins are list in Table 1. The pronounced hypsochromic shift of the original fluorescence maximum of guests in the presence of added **2** can be observed obviously in Table 1, *i.e.*, from 524 nm to 490 nm for ANS, which may be attributed to the cooperative binding of two hydrophobic cavities of **2**, since the hypsochromic shift observed upon the addition of natural β-cyclodextrin is less (524→510 nm for ANS). As shown in Fig. 1A, the fluorescence intensity of ANS was greatly enhances upon stepwise addition of bridged bis(β-cyclodextrin) **2**, indicating that the reaction of the cyclodextrin **2** and ANS has formed the host-guest inclusion complex.

Assuming 1:1 stoichiometry for the inclusion complexation of guest dyes (G) with cyclodextrins (H), where the two cyclodextrin moieties in bridged bis(β-cyclodextrin) are treated as a single unit, the complexation can be expressed by Eq. (1).

$$H + G \overset{K_s}{\rightleftharpoons} H \cdot G \qquad (1)$$

**Table 1** Emission peak changes of four guest dyes upon the addition of hosts **1** and **2**

| Guest | $c$ (μmol·$L^{-1}$) | Host | $\lambda_{ex}$ (nm) | $\lambda_{em}$ (nm) |
|---|---|---|---|---|
| ANS | 10 | None | 350 | 524 |
| | | β-CD | | 510 |
| | | **2** | | 490 |
| TNS | 10 | None | 350 | 496 |
| | | β-CD | | 483 |
| | | **2** | | 438 |
| AR | 10 | None | 490 | 561 |
| | | β-CD | | 553 |
| | | **2** | | 557 |
| RhB | 5 | None | 520 | 575 |
| | | β-CD | | 573 |
| | | **2** | | 573 |

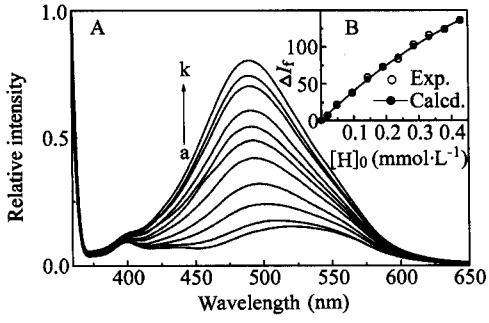



**Figure 1** (A) Fluorescence spectral changes of ANS (10.1 μmol·$L^{-1}$) upon the addition of bridged bis(β-cyclodextrin) **2** in phosphate buffer solution (pH = 7.2) at 25 ℃; the concentration of **2** (from a to k): 0, 0.02, 0.05, 0.10, 0.14, 0.19, 0.24, 0.29, 0.33, 0.38 and 0.43 mmol·$L^{-1}$, respectively; excitation at 350 nm. (B) Least-squares curve-fitting analyses for the above inclusion complexation.

The stability constants ($K_s$) of the inclusion complex formed can be calculated from the analysis of the sequential changes in fluorescence intensity ($\Delta I_f$) at varying host concentrations by using a non-linear least squares curve-fitting method according to the Eq. (2).[17]

$$\Delta I_f = \frac{1}{2}\{\alpha([H]_0 + [G]_0 + 1/K_s) \pm \sqrt{\alpha^2([H]_0 + [G]_0 + 1/K_s)^2 - 4\alpha^2[H]_0[G]_0}\} \qquad (2)$$

Here $[G]_0$ and $[H]_0$ refer to the total concentration of the organic dyes and host cyclodextrins, respectively; $\alpha$ is the proportionality coefficient, which may be taken as a sensitivity factor for the fluorescence change upon complexation.

For all host compounds examined, the $\Delta I_f$ values as a function of $[H]_0$ give excellent fits, verifying the validity of the 1:1 complex stoichiometry as assumed above. The typical curve-fitting analyses result for the inclusion complexation of host **2** with ANS is shown in Fig. 1B, where no serious deviations are found. When repeated measure-

ments were made, the $K_s$ value was reproducible within an error of ±5%, which corresponds to an estimated error of 0.15 kJ/mol in the free energy change of complexation ($-\Delta G°$). The complex stability constants ($K_s$) and the Gibbs free energy changes ($-\Delta G°$) obtained are listed in Table 2.

**Table 2** Stability constants ($K_s$) and Gibbs free energy changes ($-\Delta G°$) for the inclusion complexation of hosts (**1**—**3**) with four guest dyes at 25 ℃ in phosphate buffer solution (pH = 7.2)

| Guest | Host | $K_s$ ($L \cdot mol^{-1}$) | lg $K_s$ | $-\Delta G°$ (kJ/mol) | Ref. |
|---|---|---|---|---|---|
| ANS | **1** | 103 | 2.01 | 11.5 | 18 |
| TNS | | 3670 | 3.56 | 20.3 | 18 |
| AR | | 2630 | 3.42 | 19.5 | 10 |
| RhB | | 4240 | 3.63 | 20.7 | 10 |
| ANS | **2** | 909 | 2.96 | 16.9 | this work |
| TNS | | 7980 | 3.90 | 22.3 | this work |
| AR | | 7600 | 3.88 | 22.2 | this work |
| RhB | | 7910 | 3.90 | 22.3 | this work |
| ANS | **3** | 1280 | 3.11 | 17.7 | 14 |
| TNS | | 23800 | 4.38 | 25.0 | 14 |
| AR | | 71800 | 4.86 | 27.7 | this work |
| RhB | | 27200 | 4.43 | 25.3 | this work |

*Molecular binding ability*

It has been demonstrated that several possible weak interactions, such as van der Waals, hydrophobic interactions, as well as hydrogen bonding, contribute to the inclusion complexation behavior of cyclodextrins and most of these interactions depend on the size/shape-fit relationship of host and guest. Therefore, bridged bis(β-cyclodextrin) can significantly enhance the complex stability by the cooperative binding of one guest with two closely located hydrophobic cavities. As can be seen from Table 2, the binding ability of bridged bis(β-cyclodextrin)s **2** and **3** toward all guests investigated are increased greatly as compared with parent β-cyclodextrin, and the bridged bis(β-cyclodextrin) **3** gives the highest enhancement factor for AR as 27.3.

It is well known that the two water-soluble fluorescent dyes of ANS and TNS were barely fluoresce in aqueous solution but give intense fluorescence in a hydrophobic environment such as the cavity of cyclodextrin, and then were chosen as representative guests to investigate the inclusion complexation with bis(β-cyclodextrin)s. Though all hosts examined form less stable complex with ANS than with TNS, the enhanced molecular binding ability by the cooperative binding of cyclodextrin dimers for ANS is more remarkable than that for TNS. The enhancement factors for ANS are 8.8 (**2**) and 12.4 (**3**), which are much higher than 2.2 (**2** for TNS) and 6.5 (**3** for TNS). This may be attributed to the shape difference of linear guest TNS and bent guest ANS. Examinations of CPK molecular models indicate that ANS can penetrate only in part into the cavity of β-cyclodextrin due to the steric hindrance while TNS can be embedded deeply into the cavity of bridged bis(β-cyclodextrin) in the longitudinal direction, so the cooperative binding of the second cavity of β-cyclodextrin dimer is more effective and obvious to ANS than to TNS. These results further demonstrate that the size and shape of guest molecules are very important factors for enhancing the binding ability of bridged bis(β-cyclodextrin)s.

For further investigation of the difference of binding ability about 2,2′-bridged bis(β-cyclodextrin) (**2**) and 6,6′-bridged bis(β-cyclodextrin) (**3**), we compare the stability constants of hosts **1**—**3** with four guest dyes in Fig. 2. It can be seen clearly that host **1** displays a selectivity sequence: RhB > TNS > AR > ANS, and host **3** with the tether linked from the primary side give a different selectivity sequence, *i.e.*, AR > RhB > TNS > ANS. Comparing the relative binding ability of bridged bis(β-cyclodextrin)s, it is found that host **3** possessing the rigid conformation linked by phenylenediseleno tether gives the highest stability constant (lg $K_s$ = 4.86) for inclusion complexation with AR. One possible explanation for the strongest binding ability of host **3** is that the rigid phenylenediseleno tether leads to the relative fixed distance of two hydrophobic cavities, which makes it easy for binding with the linear guest of appropriate size. Since host **3** could include AR without large conformational change, the inclusion complexation process might be favorable from the entropic point of view.[19]

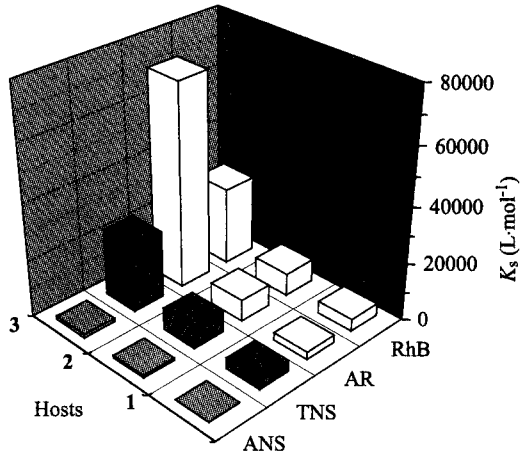



**Fig. 2** Complex stability constants ($K_s$) for the inclusion complexation of cyclodextrins **1**—**3** with four guest dye molecules.

On the other hand, host **2** linked from the secondary side and host **3** linked from the primary sides possess the same tether, but display the entirely different inclusion complexation behavior for binding with guest molecules. As illustrated in Fig. 2, host **2** shows the obviously lower binding ability toward all guests than host **3** does, and gives the almost equal $K_s$ values to guests TNS, AR and

RhB. The probably explanation for the lower molecular binding ability and molecular selectivity is that, the guest molecules penetrate into the cavities of host **2** from the larger secondary sides, making the operation of strict size/shape fitting difficult and leading to the weak interaction between guest and host **2** consequently. It is known that the primary side of cyclodextrin is more suitable with benzene ring in both size and shape than the secondary side of cyclodextrin, so if the guest just shallowly longitudinal penetrated into the hydrophobic cavity by a phenyl moiety from the secondary side, it may not achieve the strong interaction like penetration from the primary side. This statement could be proved by the different binding sequence of hosts **2** and **3** toward TNS and AR. It has been mentioned that host **3** gives the strongest binding ability toward AR, however, the highest stability constant of host **2** is obtained by the complexation with TNS. Since TNS possesses the large longitudinal size and then could be include into the cavity more deeply than AR does, the stronger interaction of TNS with host **2** linked from secondary side is obtained reasonable though it is still not as strong as that for host **3**.

It is interesting to note that the 2, 2′-trimethylenediseleno-bridged bis(β-cyclodextrin) with wider openings gives significantly higher $K_s$ values than the 6, 6′-trimethylenediseleno-bridged bis(β-cyclodextrin) as we reported previously.[14] However, the present investigation gave the entirely opposite results as the relative weak binding ability for 2, 2′-bridged bis(β-cyclodextrin) **2** linked by rigid phenylenediseleno tether. This may be attributed to that the flexible trimethylenediseleno tether can adjust two hydrophobic cavities to fit in with the size and shape of guest, which makes the guest molecules to be deeply embedded into the cavities from the secondary side of 2, 2′-bridged bis(β-cyclodextrin) and form the stable inclusion complex.

### 2D NMR *spectra*

2D NMR spectroscopy is an important and effective method to investigate the interaction between host cyclodextrins and guest molecules, when two protons are located closely enough, a NOE cross-peak between the relevant protons can be produced in NOESY or ROESY spectrum. In our previous work,[12] we have confirmed the cooperative sandwich binding mode of bis(β-cyclodextrin) with RhB. In order to further investigate the inclusion complexation behavior and deduce the molecular recognition mechanism of fluorescent dyes by bridged bis(β-cyclodextrin), the 2D NMR experiment has been performed. Fig. 3 gives the $^1$H NOESY spectrum of an equimolar mixture of **2** with RhB (5 mmol·L$^{-1}$ each) and three clear NOE cross-peaks are shown as peaks A, B and C. Peaks A illustrates the interaction between the H-3 and H-5 of cyclodextrin and the methyl protons of diethylamino fragments in RhB. The cross-peaks between the H-3 and H-5 of cyclodextrin and the aromatic protons of diethylaminophenyl groups in RhB, and the cross-peaks between the aromatic protons on the benzylenediseleno tether of host **2** and the aromatic protons of the benzoate moiety in RhB are marked as peaks B and peaks C, respectively. All these NOE correlative signals confirmed the sandwich binding mode, that is to say, the diethylaminophenyl groups of RhB are accommodated in the cavities from the secondary side of β-cyclodextrin. However, compared with peaks A and B, peaks C is not strong enough, which indicates that the benzylenediseleno tether of **2** gives much little contribution in the binding progress with RhB. Therefore, the moderate stability constants of host **2** with guests seem to be reasonable, which is consistent with the results obtained by spectral titration.

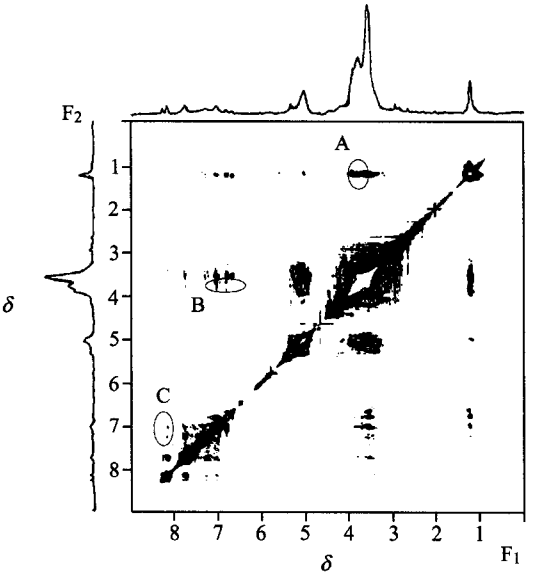



**Fig. 3** $^1$H NOESY spectrum (300 MHz) of a mixture of **2** with RhB ([**2**] = [RhB] = 5 mmol·L$^{-1}$) in $D_2O$ at 298 K.

## Conclusion

In conclusion, the molecular recognition behavior of 2, 2′-*phenylene* diseleno-bridged bis(β-cyclodextrin) linked from secondary side for some fluorescent dyes are compared with that of 6, 6′-*phenylene* diseleno-bridged bis(β-cyclodextrin) linked from primary side and the opposite binding ability sequence is obtained relative to our previous report, indicating that the flexibility of the organoselenium tether possesses the important contribution to the inclusion complexation. The rigid *phenylene* diseleno linker appears to help fixed the conformation of bridged bis(β-cyclodextrin) rather than to give a new binding site, which is further demonstrated by $^1$H NOESY spectrum. Therefore, the functional tethers attached to the primary or secondary side of two β-cyclodextrins can significatively alter the molecular binding ability and is taken as a useful tool for designing higher selective bridged bis(β-cyclodextrin)s with specific model substrate.

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(E0301264 LU, Y. J.; LU, Z. S.)