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Efficient fluorescent sensors of oligopeptides by dithiobis(2-benzoylamide)-bridged bis(β-cyclodextrin)s: structure in solution, binding behavior, and thermodynamic origin

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Abstract—Two 6,6′-bis(β-cyclodextrin)s linked by 2,2′-dithiobis[2-(benzoylamino)ethyleneamino] and 2,2′-dithiobis[2-(benzoylamino)diethylenetriamino] bridges (**1** and **2**) have been synthesized as cooperative multipoint recognition receptor models for non-aromatic oligopeptides. Their structures in solution and inclusion complexation mechanism are comprehensively investigated by means of circular dichroism, 2D NMR spectra and temperature-dependent fluorescence titrations. The results show that the cooperative 'host-linker-guest' binding mode and the extensive desolvation effect jointly contribute to the guest-induced fluorescence enhancement of bis(β-cyclodextrin)s. Further examinations on the binding behavior of hosts **1**–**2** with a series of di- and tri-peptides demonstrate that bis(β-cyclodextrin) **1** can recognize not only the size/shape of oligopeptides but also the dipeptide sequence, giving an exciting residue selectivity up to 37.5 for Gly-Gly-Gly/Glu-Glu pair and a high sequence selectivity up to 5.0 for Gly-Leu/Leu-Gly pair. These fairly good selectivities are discussed from the viewpoint of cooperative binding, multiple recognition and induced-fit interactions between host and guest.

1. Introduction

Possessing two appropriately located hydrophobic cyclodextrin (CyD) cavities in the same molecule, bis(β-CyD)s feature very high binding ability and molecular selectivity for specific ditopic guests through the cooperative two-point recognition, and thus have been employed successfully in several areas of science and technology as an excellent model system mimicking substrate-specific interaction of enzymes.[1] Hence, the molecular recognition of substrate (guest) by bis(β-CyD)s tethered with a functional linker group becomes one of the most crucial current topics in supramolecular chemistry and biochemistry.[2] Recently, a variety of dimeric CyDs with a considerable structural diversity have been synthesized to understand the recognition process controlled by the simultaneous operation of several non-covalent interactions and also to gain insights into the factors governing the inclusion complexation behavior of bridged CyD dimers.[1–9] Among them, the development of CyD-based receptors for residue- and sequence-selective recognition of peptides is one of the most challenging tasks,[1a,6c,10,11] since the peptide recognition by synthetic receptors is known to mediate versatile protein–protein interactions, endowing specific biochemical functions.[12,13] However, works on the molecular recognition of bis(β-CyD)s are concentrated mostly on the inclusion complexation of rather simple organic guests and amino acids, and practically less attempts have been made on the recognition of non-aromatic oligopeptides, exclusive of the elegant work on oligopeptides carrying two aromatic amino acid residues for a simultaneous complexation by CyD cavities[1a,6c] and on testing a library of aromatic and non-aromatic tripeptides for binding to a bis- as well as a mono-CyD receptor.[11] Recently, Reinhoudt and co-workers reported two bridged bis(β-CyD)s tethered by photoswitchable bis(phenylthienyl)ethene moieties as potentially tunable receptor molecules.[7c] More recently, we reported the molecular recognition behavior of non-aromatic oligopeptides by bis(β-CyD)s with dithiobis(2-benzoylamide)[14] and pyridine-2′6-dicarboxamide tethers,[15] showing satisfactory sequence-selectivity for Gly-Leu/Leu-Gly and Gly-Gly-Gly/Gly-Gly pairs. To further elucidate the detailed recognition mechanism of

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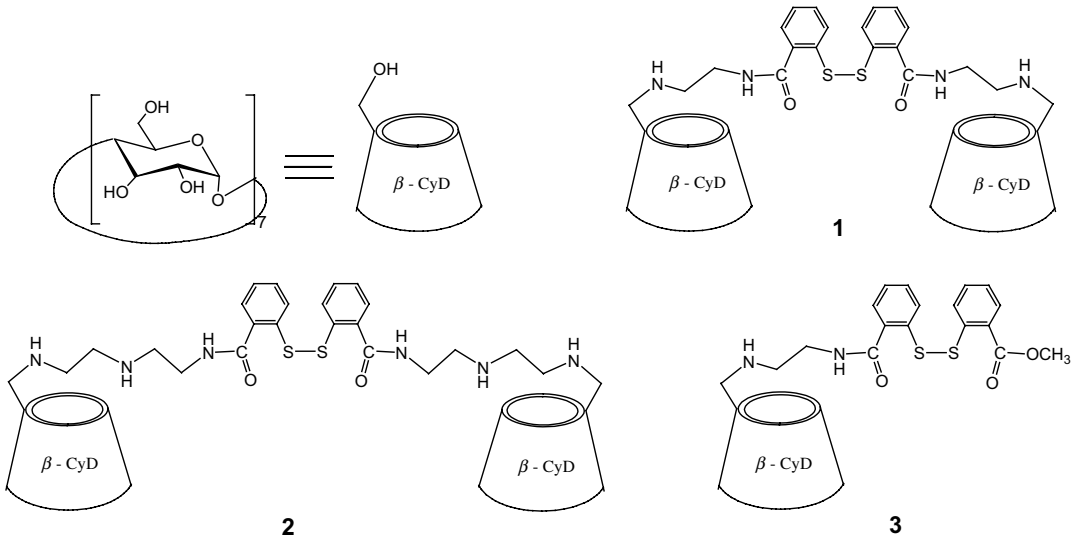

Chart 1.

functional bis(β-CyD)s toward oligopeptides, we synthesize two bis(β-CyD)s, that is, 6,6′-[2,2′-dithiobis[2-(benzoylamino)ethyleneamino]]-bridged bis(β-CyD) (**1**) and 6,6′-[2,2′-dithiobis[2-(benzoylamino)diethylenetriamino]]-bridged bis(β-CyD) (**2**) as ditopic molecular receptors, and a mono-modified β-CyD that contains one CyD unit substituted with a tether with one end group protected by a methyl group instead of the second CyD, that is, mono[6-(2,2′-dithio-1′-benzoylmethylester-1-benzoylaminoethyleneamino)-6-deoxy]-β-CyD (**3**), as the reference (Chart 1). Their structures in solution and binding behavior toward a series of oligopeptides (Leu-Gly, Gly-Leu, Glu-Glu, Met-Met, Gly-Gly, Gly-Pro, Gly-Gly-Gly, Glu-Cys-Gly) are investigated by means of circular dichroism, fluorescence, and 2D NMR spectra in an aqueous phosphate buffer solution (pH 7.20). These studies will help us gain a deeper insight into the binding interaction and molecular recognition process of peptides by bis(β-CyD)s in the neutral biological environment such as serum, and consequently further their application as drug carriers.

2. Results and discussion

2.1. Structure of bis(CyD) in solution

It has been amply demonstrated that the inclusion of achiral chromophore in a chiral environment such as CyD cavity can produce the induced circular dichroism (ICD) signals at the wavelengths absorbed by the chromophore.[16] Therefore, in order to investigate the original conformation of hosts **1**–**3**, their circular dichroism spectra are measured at a low concentration of 1.0×10^{-4} M in an aqueous buffer solution of pH 7.20. Seen from Figure 1, possessing the dithiobisbenzoylamide chromophore and chiral CyD unit(s), hosts **1**–**3** display appreciable ICD signals in the absence of guest oligopeptides. Bis(β-CyD) **1** shows a weak positive Cotton effect peak at 290 nm ($\Delta\varepsilon = 0.10\ M^{-1}\ cm^{-1}$) for the ^1L_b transition and a relatively strong positive Cotton

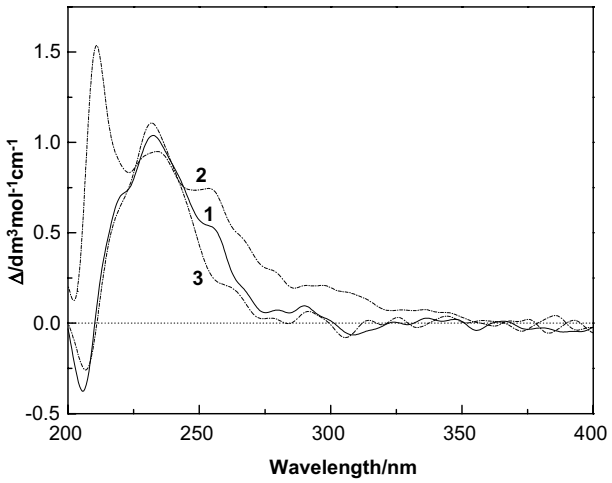

Figure 1. Circular dichroism spectra of hosts **1**–**3** in an aqueous buffer solution of pH 7.20 at 25 °C.

effect at 232 nm ($\Delta\varepsilon = 1.04\ M^{-1}\ cm^{-1}$) for the ^1L_a transition of aromatic group in 2,2′-dithiobisbenzoylamide linker. As a homologue of **1**, host **2** gives the similar ICD signals; that is, two positive Cotton effect peaks at 234 nm ($\Delta\varepsilon = 0.95\ M^{-1}\ cm^{-1}$) and 296 nm ($\Delta\varepsilon = 0.21\ M^{-1}\ cm^{-1}$), respectively, assigned to ^1L_a and ^1L_b bands. In the control experiment, mono-modified β-CyD **3** also displays similar ICD spectrum to that of **1**. According to the generally accepted empirical rule for the ICD of aryl-CyD,[17] the sign of ICD signal depends on the orientation of the transition dipole moment of the chromophore with respect to the CyD axis. The electronic transition parallel to the CyD axis gives a positive ICD signal, whereas perpendicular transitions gives a negative signal, but this situation is reversed for a chromophore located just outside the CyD cavity. So we can deduce that the aromatic chromophore in 2,2′-dithiobisbenzoylamide group of hosts **1**–**3** penetrates shallowly into the CyD cavities. Moreover, the similar ICD shape and intensity between

bis(CyD)s **1**–**2** and mono-modified **3** indicate that the aromatic chromophore in hosts **1**–**2** is only self-included in one CyD cavity of bis(CyD).

The self-included conformation of bis(CyD)s **1**–**2** is also verified by 2D NMR experiments, since one can conclude that two protons are closely located in space if an NOE correlation is detected between the relevant protons in the NOESY or ROESY spectrum. From the ROESY experiments (seeing supplementary data), we can find the clear correlations between the aromatic protons in the linker group and the interior proton (H-3, H-5) of CyD cavity as well as the correlations between the ethylene protons in the linker group and the interior protons of CyD. These relevant signals indicate that both the aromatic phenyl group and the non-aromatic ethylenediamino group are accommodated in the CyD cavity, which strongly support the self-included geometry of bis(CyD) deduced from the ICD analysis.

2.2. Inclusion complexation stoichiometry

The stoichiometry for the inclusion complexation of bis(β-CyD)s with representative oligopeptide is determined by the continuous variation method. In the concentration range examined, all of the plots for bis(β-CyD) units in the Job's experiments of bis(β-CyD)/oligopeptide systems show the maximums at a molar fraction of 0.5, indicating the 1:1 sandwich complexation between host bis(β-CyD) and guest oligopeptide.

2.3. Fluorescence titration

For a more quantitative assessment of the inclusion complexation behavior of bis(CyD)s, spectral titration experiments of hosts **1**–**3** with guest oligopeptides are performed in aqueous buffer solution (pH 7.20) at 25 °C. An interesting phenomenon is that, although the UV and circular dichroism spectra of hosts **1**–**2** display no appreciable change with the addition of guests, their fluorescence intensity obviously increases with the gradual addition of guest oligopeptide, as can be seen from Figure 2. Since the emission intensity of dithiobisbenzoyl fluorophore is sensitive to the microenvironment change, being stronger in a hydrophobic microenvironment than in a hydrophilic one, the above results suggest that the dithiobisbenzoyl moiety is located in a more hydrophobic microenvironment after inclusion complexation with guest oligopeptide. According to our conclusion drawn from the ICD experiments, the dithiobisbenzoyl chromophore in bis(CyD) is only self-included in one CyD cavity. Therefore, in the host–guest inclusion complexation, the guest oligopeptide will penetrate into another CyD cavity to form 'host-linker-guest' co-inclusion complex and thus push the dithiobisbenzoyl chromophore deeply into the hydrophobic CyD cavity, which consequently lead to the enhanced fluorescence of bis(CyD).

Besides the 'host-linker-guest' binding mode, the desolvation effect upon inclusion complexation also contributes to the fluorescence enhancement of bis(CyD). Examinations on the thermodynamic behavior of bis-

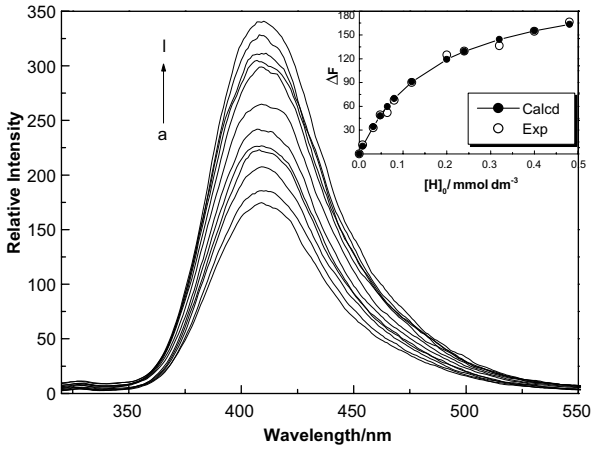

Figure 2. Fluorescence spectral changes of bis(β-CyD) **1** (8 μM) and the nonlinear least-square analysis (inset) of the differential intensity (ΔF) used to calculate the complex stability constant (K_S) upon addition of Gly-Pro (0–480 μM; traces a–l) in an aqueous buffer solution of pH 7.20 at 25 °C; excitation at 295 nm.

(CyD)s by temperature-dependent fluorescence titration experiments show that all of the inclusion complexation between bis(CyD)s examined and guest oligopeptides are entirely driven by the favorable entropic gain ($\Delta S°$), which overwhelms the endothermic enthalpic changes ($\Delta H°$). Since the host–guest association process always leads to the loss of conformational freedom, which is inherently accompanied by the unfavorable entropic loss, the positive entropic gain observed in the inclusion complexation of bis(CyD) with oligopeptide should mainly come from the extensive desolvation effect of bis(CyD) upon inclusion complexation. This effect will force the solvent molecules (in the present case, H_2O) distant from the bis(CyD) hosts and thus increase the microenvironment hydrophobicity around the dithiobisbenzoyl fluorophore, which consequently results in the enhanced fluorescence intensity of bis(CyD) upon inclusion complexation with guest oligopeptide.

2.4. Peptide binding ability and selectivity

Validating the 1:1 host–guest inclusion complexation stoichiometry, the complex stability constants (K_S) can be calculated from the analysis of the sequential changes of fluorescence intensity (ΔF) at various guest concentrations, using a nonlinear least-square method according to the curve fitting.[18] For each host–guest pair examined, the ΔF values are plotted as a function of $[H]_0$ to give an excellent fit. The experimental data do not show any significant deviations from the theoretical curve in each case. In the repeated measurements, the K_S values are reproducible within an error of ±5%, which corresponds to an estimated error of ca. 0.15 kJ/mol in free energy change. The K_S values obtained are listed in Table 1, along with the Gibbs free energy changes ($-\Delta G°$). It is also significant to note that, lacking the second CyD cavity as compared with bis(CyD), mono-CyD **3** shows no appreciable fluorescence changes upon addition of guest oligopeptide, which disables the

Table 1. Complex stability constants (K_S) and Gibbs free energy changes ($-\Delta G°$) for 1:1 inclusion complexation of oligopeptides with β-CyD derivatives **1**–**3** in aqueous buffer solution of pH 7.20 at 25 °C

Host	Guest	K_S/M^{-1}	$-\Delta G°/kJ\ mol^{-1}$	
1	Glu-Glu	590 ± 50	15.8	[a]
	Gly-Gly	1680 ± 80	18.4	[a]
	Leu-Gly	3300 ± 200	20.1	[a]
	Met-Met	5600 ± 250	21.4	[a]
	Gly-Pro	6200 ± 300	21.7	[a]
	Gly-Leu	16600 ± 800	24.1	[a]
	Gly-Gly-Gly	22100 ± 1100	24.8	[b]
	Glu-Cys-Gly	8840 ± 200	22.5	[b]
2	Glu-Glu	[c]	[c]	[b]
	Gly-Gly	25100 ± 1300	25.1	[b]
	Leu-Gly	13600 ± 800	23.6	[b]
	Met-Met	3250 ± 150	20.1	[b]
	Gly-Pro	6530 ± 350	21.8	[b]
	Gly-Leu	10900 ± 600	23.0	[b]
	Gly-Gly-Gly	44200 ± 2500	26.5	[b]
	Glu-Cys-Gly	9070 ± 200	22.6	[b]
3	oligopeptides	[c]	[c]	[b]

[a] Ref. 14.
[b] This work.
[c] The spectral changes are too weak to calculate the K_S value.

calculation of the binding constants. This phenomenon further validates the importance of the second CyD cavity in bis(CyD), since the cooperative 'host-linker-guest' binding mode working between host and guest requires two CyD cavities, respectively, including the linker group and guest molecule.

From the observations described above, we can find that an important characteristic of molecular recognition of these dithiobisbenzoylamide-bridged bis(CyD)s toward oligopeptides is the cooperative 'host-linker-guest' binding mode, where the guest oligopeptide is embedded into one hydrophobic CyD cavity while the linker group partly self-included in the other cavity. According to this binding mode, several interactions will simultaneously work between host and guest. Besides the van der Waals and hydrophobic interactions between CyD cavity and guest oligopeptide, the oligoethylenediamino fragment in the linker group located near the accommodated guest will provide the additional hydrogen-bond interactions with guest oligopeptide. Attributing to the cooperative effect of these contributions, bis(CyD)s **1**–**2** give moderate to high binding ability toward aliphatic oligopeptide with the K_S values varying from 590 to 44200 M^{-1}. Seen from Table 1, the K_S values increase in the following orders:

for **1**: Glu-Glu < Gly-Gly < Leu-Gly < Met-Met < Gly-Pro < Glu-Cys-Gly < Gly-Leu < Gly-Gly-Gly
for **2**: Met-Met < Gly-Pro < Glu-Cys-Gly < Gly-Leu < Leu-Gly < Gly-Gly < Gly-Gly-Gly

Among the guest oligopeptides examined, both of bis(CyD)s **1**–**2** give the strongest association with tripeptide Gly-Gly-Gly. This may be attributed to the strict size/shape-fit between host and guest. Possessing a relatively long skeleton and less branched chains, Gly-Gly-Gly is able to fully enjoy the cooperative multipoint binding of bis(CyD) as compared with other guest oligopeptides. Moreover, the stronger hydrogen-bond interactions between two carbonyl groups in Gly-Gly-Gly and the oligoethylenediamino groups in the linker of bis(CyD)s further promote the host–guest inclusion complexation. These factors jointly contribute to the strongest binding ability of bis(CyD)s toward Gly-Gly-Gly. However, the poor hydrophobicity of Glu-Glu arising from the presence of three hydrophilic carboxyl groups in the molecule dramatically reduces its hydrophobic interactions with CyD cavity and thus results in the smallest K_S value. Owing to this multiple recognition mechanism involving size/shape-fit, hydrogen-bond, and hydrophobic interactions, bis(CyD) **1** affords fairly high residue selectivity toward Gly-Gly-Gly/Glu-Glu pair ($K_S^{(Gly\text{-}Gly\text{-}Gly)}/K_S^{(Glu\text{-}Glu)} = 37.5$).

Another intriguing phenomenon is the high sequence sele ctivity of bis(CyD) toward Gly-Leu/Leu-Gly pair ($K_S^{(Gly\text{-}Leu)}/K_S^{(Leu\text{-}Gly)} = 5.0$), which may point to the different host–guest binding mode upon inclusion complexation. For **1**/Gly-Leu system, the ROESY spectrum (Fig. 3a) shows the correlations (peak D) between the isobutyl protons of Gly-Leu and the ethylene protons in the linker group of **1**, which indicates that the Leu residue in guest oligopeptide and ethylene unit in **1** are located in short distance. Moreover, since these two groups (isobutyl group in Gly-Leu and ethylene group in **1**) also show correlations (peaks B and C) with the interior protons (H-3/H-5) of CyD cavity, we can deduce that the guest oligopeptide and oligoethylenediamino group in **1** are simultaneously included in the same CyD cavity. On the other hand, the aromatic protons in the linker group show no correlations with the isobutyl protons of Gly-Leu, but exhibit the clear correlation (peak A) with the H-5 protons of CyD cavity. Since H-3 protons are located near the wide side of CyD cavity, while H-5 protons near the narrow side, we can deduce that the phenyl unit in the linker group is self-included in the another CyD cavity from the narrow side. This result not only validates the 'host-linker-guest' binding mode as described above, but also further rationalizes the high binding ability of **1** toward Gly-Leu. Since the guest Gly-Leu and oligoethylenediamino group in **1** are closely located, the electrostatic attraction between the protonated linker NH_2^+ and Leu–CO_2^- will provide the additional binding interactions between host and guest, which consequently favor the host–guest inclusion complexation to some extent.

Different from the **1**/Gly-Leu system, the ROESY spectrum of **1**/Leu-Gly system (Fig. 4) displays the correlations (peaks A) between the isobutyl protons of Leu-Gly and H-3/H-5 of CyD cavity, while no other NOE correlations between host and guest can be found. Moreover, it can be seen from the peaks A that the methenyl proton in isobutyl group merely correlates with the H-3 protons of CyD, but the methyl protons correlate with both the H-3 and the H-5 protons of CyD. In addition, the correlation (peaks B) between the ethylene protons in the linker group and the H-5 protons of CyD is also observed, indicating that the

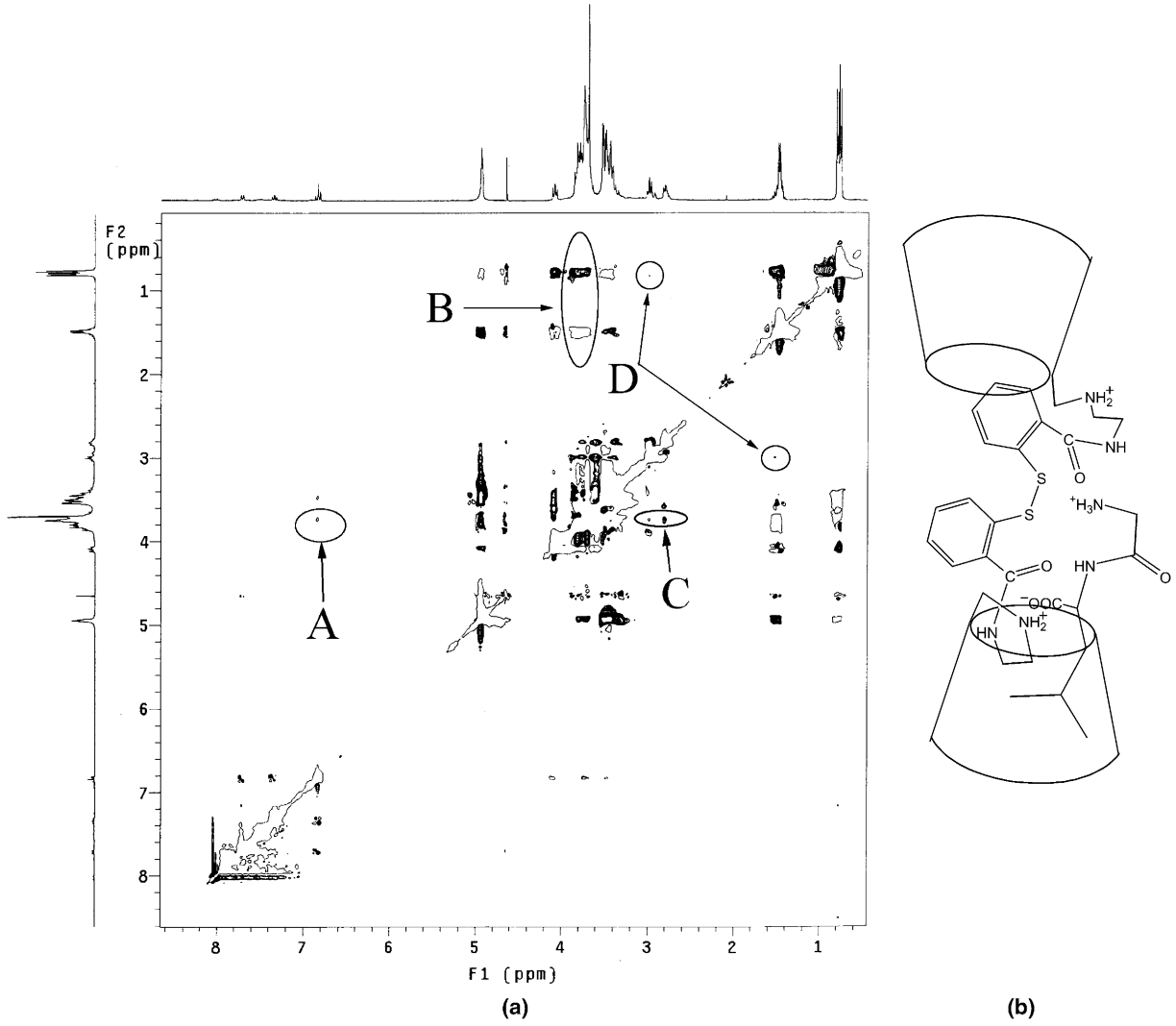

Figure 3. ROESY spectrum of **1**/Gly-Leu system ([**1**] = [Gly-Leu] = 2.0 mM) in pD 7.20 buffer solution.

tether group is partially self-included into one CyD cavity. Therefore, we can deduce a possible binding mode as illustrated in Figure 4b; that is, the isobutyl group of Leu-Gly may have to enter one CyD cavity from the wide side due to the unfavorable electrostatic repulsion between the protonated amino group in the linker ($-NH_2^+-$) and the relevant charged group (NH_3^+) remaining in the amino acid residue, while the other CyD cavity of **1** is occupied by the self-included linker group. In this mode, the inclusion complexation is only supported by the van der Waals and hydrophobic interactions between CyD cavity and accommodated guest, which inevitably causes the relatively weak binding ability of **1** toward Leu-Gly.

Unexpectedly, possessing similar linker group, bis(CyD) **2** displays fairly weak sequence selectivity toward Gly-Leu/Leu-Gly pair ($K_S^{(Gly\text{-}Leu)}/K_S^{(Leu\text{-}Gly)} = 0.8$). This should be rationalized from the ROESY analyses. For **2**/Gly-Leu system, Figure 5a shows that the H-3/H-5 protons of CyD give the clear correlations with the aromatic protons in the linker group (peaks A) as well as the isobutyl protons of Gly-Leu (peaks B), indicating the cooperative 'host-linker-guest' binding mode. Further analysis on peaks B shows that the methyl protons in the isobutyl group of Gly-Leu give stronger correlations with the H-5 protons of CyD than those with the H-3 protons (peaks B1), but the methenyl proton in the isobutyl group of Gly-Leu displays stronger correlations with the H-5 protons of CyD than those with the H-3 protons (peaks B2). This indicates that the isobutyl group of Gly-Leu is included in CyD cavity from the wide side, as illustrated in Figure 5b.

Like that of **2**/Gly-Leu system, the ROESY spectrum of **2**/Leu-Gly system (Fig. 6) gives the similar correlations between the H-3/H-5 protons of CyD and the aromatic protons in the linker group (peaks A) as well as the isobutyl protons of Leu residue in Leu-Gly (peaks C). This phenomenon may point to a similar 'host-linker-guest' co-inclusion mode to the case of **2**/Gly-Leu system, and thus rationalize the similar binding constants of bis(CyD) **2** toward Gly-Leu and Leu-Gly. On the other hand, as shown in Figure 6, the ethylene protons in the linker group also display the correlations with the H-3/H-5 protons of CyD (peaks B) as well as the

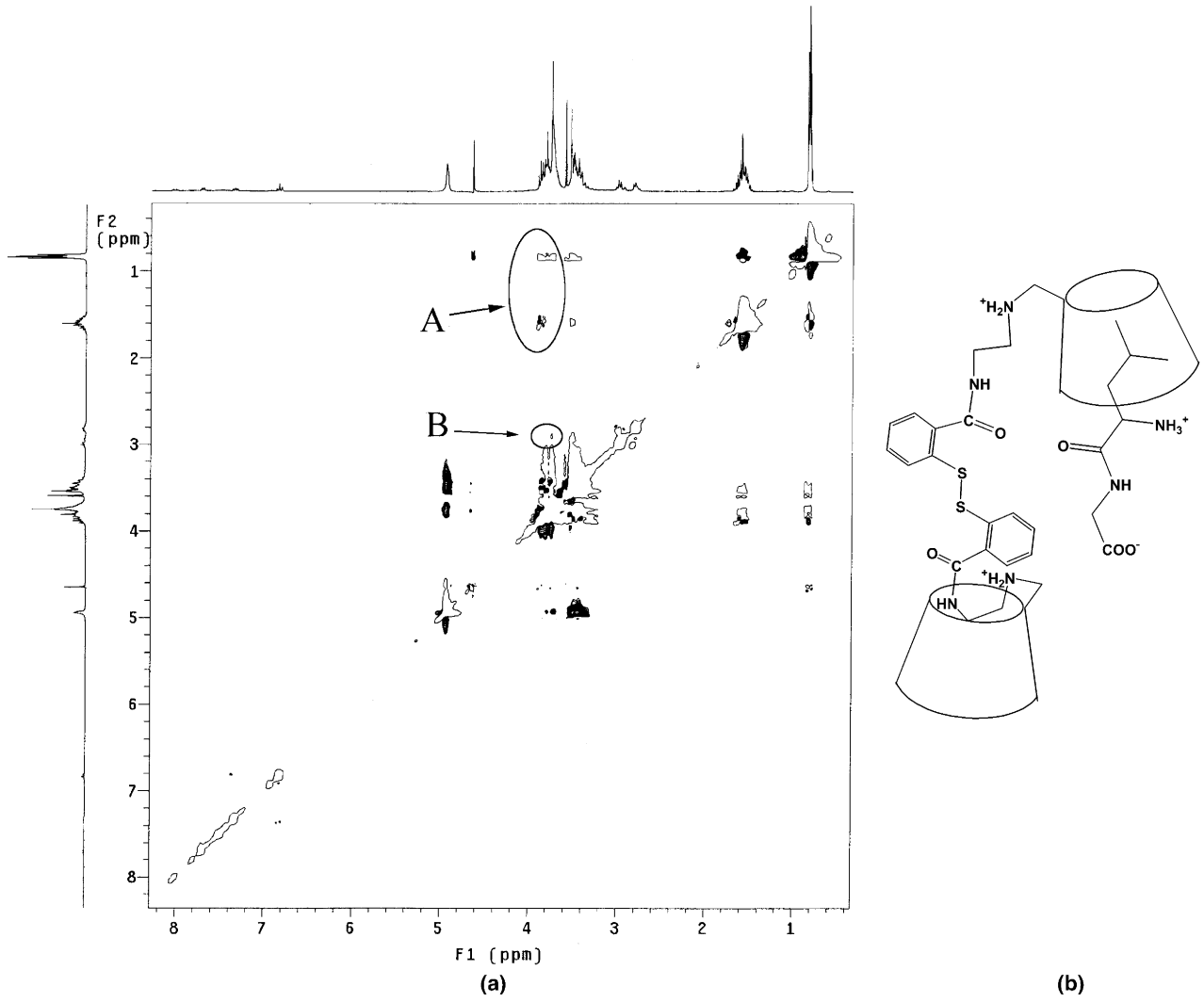

Figure 4. ROESY spectrum of **1**/Leu-Gly system ([**1**] = [Leu-Gly] = 2.0 mM) in pD 7.20 buffer.

isobutyl protons of Leu residue in Leu-Gly (peaks D). These correlations indicate that the oligoethylenediamino group in **2** is partly self-included in the CyD cavity where the guest Leu-Gly is accommodated. Therefore, the van der Waals and hydrophobic interactions between oligoethylenediamino group and Leu residue will strengthen the host–guest inclusion complexation to some extent, which subsequently leads to the slightly enhanced binding constant of **2**/Leu-Gly system as compared with that of **2**/Gly-Leu system.

In summary, we have demonstrated that the dithiobenzoylamide-bridged bis(β-CyD)s **1**–**2** are found able to serve as efficient molecular receptors for non-aromatic oligopeptides, exhibiting very high residue and sequence selectivity. Moreover, the cooperative 'host-linker-guest' co-inclusion binding mode presented in this study will help us deeply understand the 'multipoint, induced-fit' receptor–substrate interactions often observed in the biological system and provide a convenient and powerful tool for the design of efficient peptide receptor with high binding ability and sequence selectivity. We also believe that the present host design, materializing the cooperative, multipoint/multimode recognition by introducing a functional linker to bis(CyD)s, is potentially applicable in general to the complexation of homologous oligopeptides.

3. Experimental

3.1. Materials

All guest oligopeptides, that is, Leu-Gly, Gly-Leu, Gly-Pro, Met-Met, Glu-Glu, Gly-Gly, Gly-Gly-Gly, and Glu-Cys-Gly were purchased from Tokyo Peptide Institute Inc. All chemicals were reagent grade and used without further purification unless noted otherwise. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for two days and then distilled under a reduced pressure before use. Pyridine was refluxed over calcium hydride for 8 h and distilled prior to use. β-CyD of reagent grade was recrystallized twice from water and dried in vacuo at 100 °C for 24 h prior to use. Mono[6-(2-aminoethyleneamino)-6-deoxy]-β-CyD and mono[6-diethylenetriamino-6-deoxy]-β-CyD were

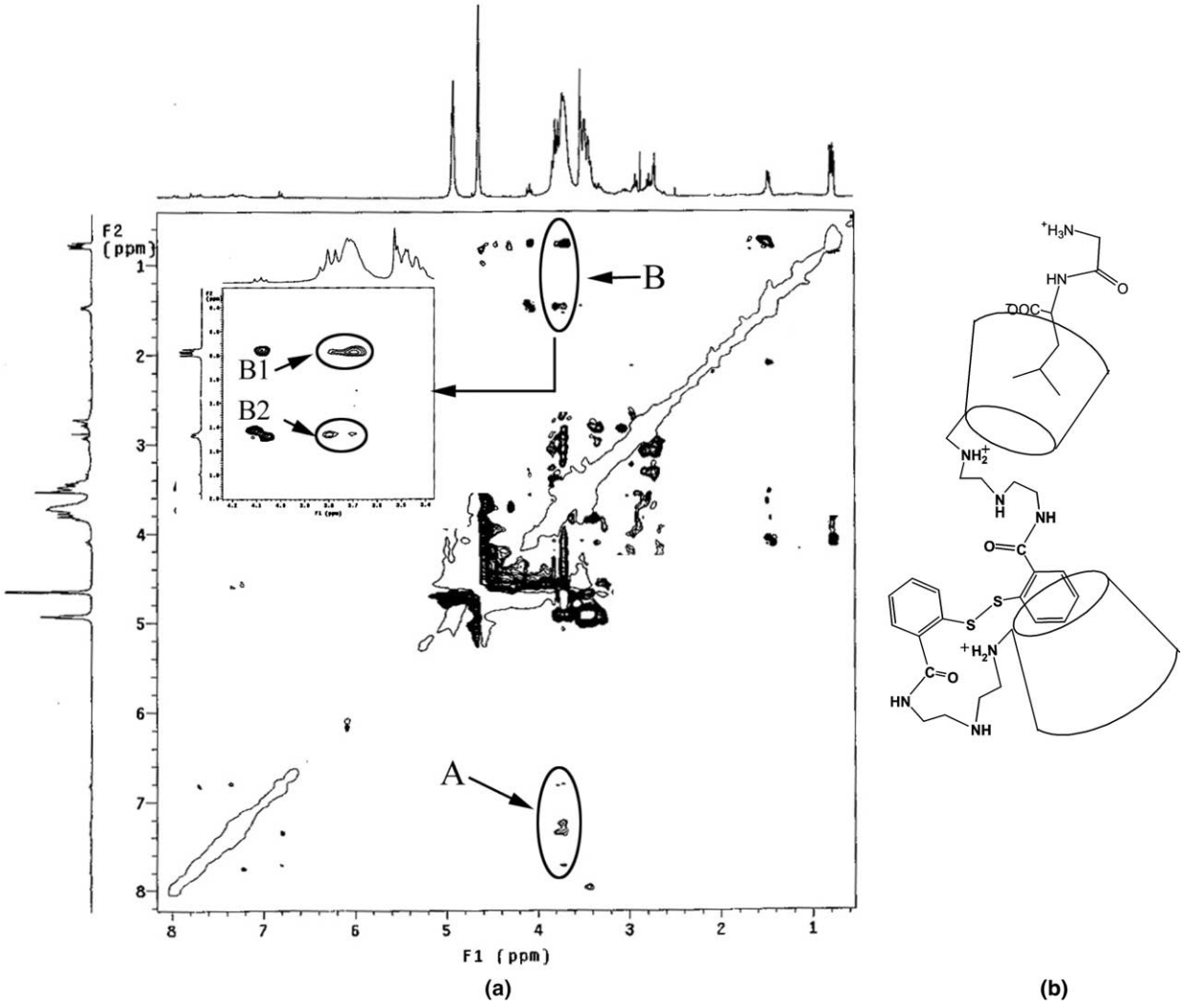

Figure 5. ROESY spectrum of **2**/Gly-Leu ([**2**] = [Gly-Leu] = 2.0 mM) in pD 7.20 buffer.

prepared according to the literature procedure.[19] Disodium hydrogen phosphate dodecahydrate (25.79 g) and sodium dihydrogen phosphate dihydrate (4.37 g) were dissolved in 1000 mL of deionized water to make a 0.10 M aqueous phosphate buffer solution of pH 7.20.

3.2. Synthesis of 6,6′-[2,2′-dithiobis[2-(benzoylamino)-ethyleneamino]]-bridged bis(β-CyD) (1)

Compound **1** was prepared from 2,2′-dithiobis(benzoic acid) and mono[6-(2-aminoethyleneamino)-6-deoxy]-β-CyD according to our recently reported procedures.[14]

3.3. Synthesis of 6,6′-[2,2′-dithiobis[2-(benzoylamino)-diethylenetriamino]]-bridged bis(β-CyD) (2)

Compound **2** was synthesized with a yield of 25% by the reaction of 2,2′-dithiodibenzoic acid and mono[6-diethylenetriamino-6-deoxy]-β-CyD according to procedures similar to those in the synthesis of **1**. ^{1}H NMR (D_2O, 300 MHz, TMS, ppm): δ 2.54–3.02 (m, 16H); 3.22–3.77 (m, 84H); 4.86 (s, 14H); 6.73–7.73 (m, Ar 8H). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 177.1, 164.9, 160.5, 133.6, 130.51, 118.6, 116.2, 102.0, 83.5, 81.1, 73.1, 72.1, 71.9, 70.1, 60.2, 49.1, 47.0, 38.5. Anal. Calcd for $C_{106}H_{168}O_{70}N_6S_2{\cdot}14H_2O$: C, 42.97; H, 6.67; N, 2.84; S, 2.16. Found: C, 43.00; H, 6.45; N, 3.05; S, 2.17.

3.4. Synthesis of mono[6-(2,2′-dithio-1′-benzoylmethyl-ester-1-benzoylaminoethyleneamino)-6-deoxy]-β-CyD (3)

Compound **3** was synthesized by the reaction of 2,2′-dithiobis(benzoic acid), mono[6-(2-aminoethyleneamino)-6-deoxy]-β-CyD, and methanol, according to our recently reported procedures.[14]

3.5. Measurements

Elemental analyses were performed on a Perkin–Elmer-2400C instrument. NMR experiments were recorded on a Varian Mercury VX300 instrument or a Bruker AV600 spectrometer. FT-IR spectra were obtained on a Nicolet FT-IR 5DX spectrometer. Circular dichroism and UV–vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a JASCO J-715S spectropolarimeter and a Shimadzu UV-2401PC

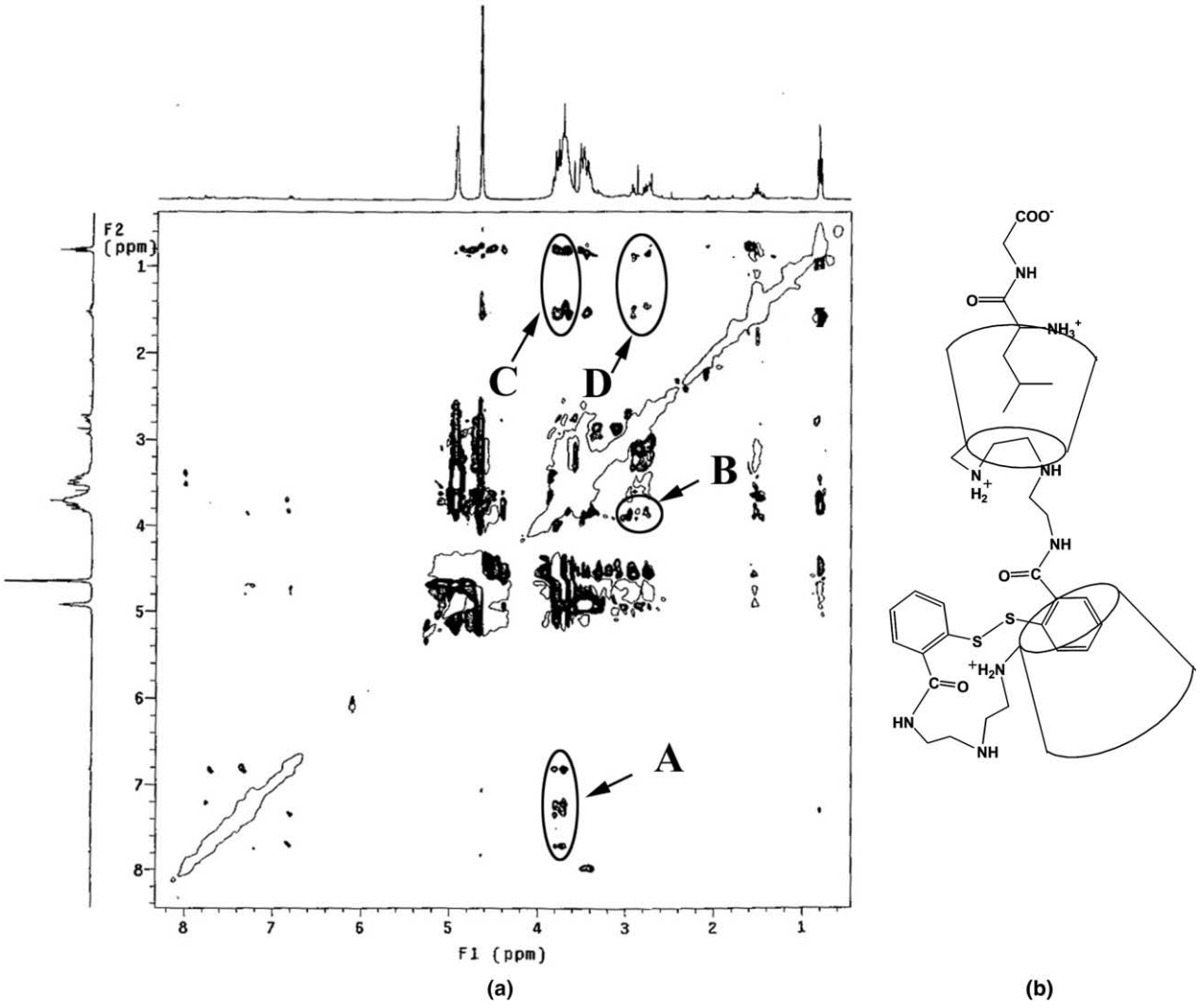

Figure 6. ROESY spectrum of **2**/Leu-Gly ([**2**] = [Leu-Gly] = 2.0 mM) in pD 7.20 buffer.

spectrophotometer equipped with a PTC-348WI temperature controller to keep the temperature at 25 °C, respectively. Fluorescence spectra were measured in a conventional quartz cell ($10 \times 10 \times 45$ mm) at several temperatures varying from 25 to 45 °C on a JASCO FP-750 spectrometer equipped with a constant-temperature water bath, with the excitation and emission slits of 5 nm width for all measurements.

3.6. ROESY experiments

Two-dimensional rotating frame nuclear Overhauser effect spectroscopy (ROESY) was performed on a Bruker AV600 spectrometer at 600.13 MHz. A Bruker standard sequence with water suppression (roesyphpr) was necessary to remove the signal of residual HOD and to be able to observe weak intermolecular NOE interactions. The data consisted of eight scans collected over 2048 complex points and for a spectral width of 6127 Hz. A mixing time of 200 ms, a repetition delay of 1.5 s, an acquisition time of 0.167 s, and a 90° pulse width of 7.95 μs at −3 dB power attenuation were used. The data were zero-filled to 1024×1024 points and processed by applying a $\pi/2$ shifted Q-sine window in both dimensions. Small cross-peaks were neglected when their magnitude was close to that of noise.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.11.042.

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