# Formation of inclusion complexes between dimers of (*R*)-3-hydroxybutanoic acid and β-cyclodextrin: thermodynamic study of the complexation and conformational analysis of the complexed dimers

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The formation of inclusion complexes of di[(*R*)-3-hydroxybutanoate] (Dimer **1**) and the methyl ester of di[(*R*)-3-hydroxybutanoate] (Dimer **2**) with β-cyclodextrin (β-CD) was studied by NMR spectroscopy. The stoichiometry of the complexation was 1 : 1 (host : guest). Thermodynamic analysis revealed that the complex formation is enthalpically favorable, but entropically unfavorable. Dimer **1** forms hydrogen bonds with β-CD more frequently than Dimer **2** because **1** has two hydroxy groups. Conformational analysis of the 3HB (3-hydroxybutanoate) dimers in the complexes indicates that they have extended (*trans*) forms. In contrast, in solution without β-CD, the end of both Dimers **1** and **2** takes a sickle (*gauche*) shape due to formation of intermolecular hydrogen bonds.

## Introduction

Poly[(*R*)-3-hydroxybutanoate] [P(3HB)] is an optically active biopolyester synthesized as a storage material for carbon and energy in many prokaryotic microorganisms.[1,2] Besides storage properties, a trace of narrow-disperse low-molecular-weight P(3HB) of physiological significance was found in a variety of prokaryotic and eukaryotic organisms,[3,4] and even in human blood plasma.[5] Low-molecular-weight P(3HB) always appears to be complexed with other specific large molecules.[6] Although the mechanism is still not clear, there is no doubt that molecular recognition plays an important role in the physiological processes involving P(3HB).[3c]

Oligomers of 3HB have attracted much attention in terms of their synthesis, structures, and functions.[7–9] We recently studied conformational structures of dimers and some oligomers of 3HB in solution, and showed that the monomer unit adjacent to the terminal hydroxy group has a different conformational distribution due to intramolecular hydrogen bonding between the hydroxy and carbonyl groups.[10]

Cyclodextrins (CDs) are a series of doughnut-shaped cyclic oligosaccharides composed of 6, 7, and 8 D-(+)-glucose units linked by α-1,4-linkages, and named α-, β-, and γ-CD, respectively. Their hydrophobic cavities are able to bind various molecules to form inclusion complexes.[11] They have been extensively studied as models to mimic enzyme activity and to provide understanding of the mechanism of molecular recognition.[11,12] In a previous paper,[13] we reported that di[(*R*)-3-hydroxybutanoate] (Dimer **1**) selectively forms an inclusion complex with β-CD in aqueous solution, and carried out a thermodynamic analysis of the complex formation. The methyl ester of di[(*R*)-3-hydroxybutanoate] (Dimer **2**) was synthesized as another model compound of P(3HB). We have also found an inclusion complex between Dimer **2** and β-CD in aqueous solution. It is of great interest to learn how the complexation affects the conformations of the guest molecules. In this paper, we report the conformational analysis of Dimer **1** and Dimer **2** in the β-CD complexes, and compare the conformational structures of the 3HB dimers in the complexes with those of free 3HB dimers in solution. The thermodynamic analysis of Dimer **2**–β-CD complexation is also reported and compared with that of complexation of Dimer **1**. Fig. 1 shows the chemical structures of β-CD, P(3HB), Dimer **1**, and Dimer **2**.

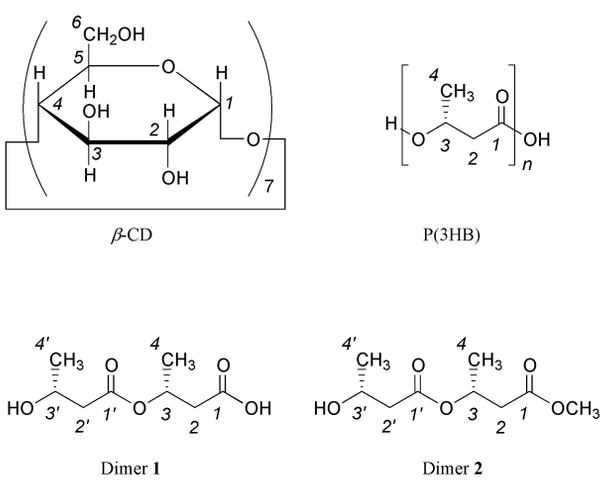


**Fig. 1** The chemical structures of β-CD, P(3HB), Dimer **1**, and Dimer **2**.

## Results and discussion

### Complex formation between Dimer 2 and β-CD

Upon addition of β-CD to a $D_2O$ solution of Dimer **2**, $^1H$ and $^{13}C$ NMR spectra of Dimer **2** were found to show changes in chemical shift and/or in resonance pattern. Fig. 2 shows $^1H$ NMR spectra of Dimer **2** in the absence and the presence of various amounts of β-CD in $D_2O$ solution. Upon addition of β-CD, resonances of H2, H3, and H3′ of Dimer **2** show a marked shift to higher magnetic fields, while those of H4 and $-OCH_3$ shift downfield, and resonance patterns of H2 and H2′ show marked changes. On the other hand, upon addition of α-CD or γ-CD to a $D_2O$ solution of Dimer **2**, both $^1H$ and $^{13}C$ NMR spectra of Dimer **2** were found not to show marked changes in chemical shift or in resonance pattern. The results

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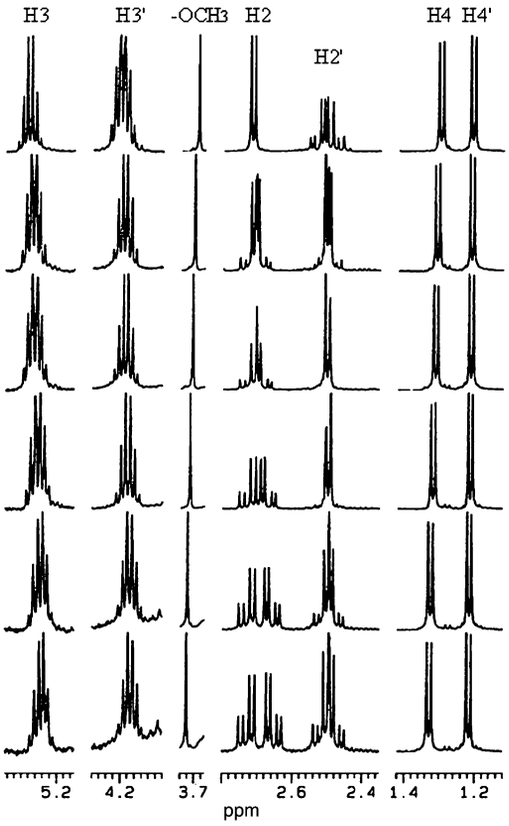


**Fig. 2** 500-MHz $^1$H NMR spectra of Dimer **2** in the absence and the presence of β-CD in $D_2O$ at 27 °C. The concentrations for β-CD–Dimer **2** are (from top to bottom) 0 : 20.0, 5.0 : 15.0, 6.7 : 13.3, 10.0 : 10.0, 13.3 : 6.7, 15.0 : 5.0 mmol dm$^{-3}$.

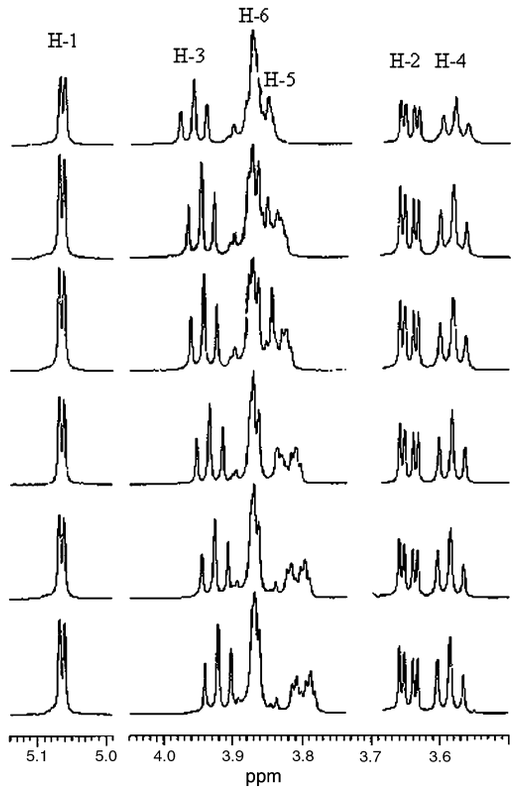


**Fig. 3** 500-MHz $^1$H NMR spectra of β-CD in the absence and the presence of Dimer **2** in $D_2O$ at 27 °C. The concentrations for Dimer **2**–β-CD are (from top to bottom) 0 : 20.0, 5.0 : 15.0, 6.7 : 13.3, 10.0 : 10.0, 13.3 : 6.7, 15.0 : 5.0 mmol dm$^{-3}$.

indicate that Dimer **2** selectively forms a complex with β-CD in aqueous solution, the cavity size of which is a fit for the dimers of 3HB. As we reported in a previous paper,[13] monomeric 3HB does not form inclusion complexes with α-, β-, or γ-CD at all. Therefore, the complex formation between Dimer **2** and β-CD is of high selectivity, and the size fitting between the dimers of 3HB and β-CD plays an important role in the inclusion complexation.

Fig. 3 shows the $^1$H NMR spectra of β-CD in the absence and the presence of Dimer **2** in $D_2O$ solution. Upon addition of Dimer **2** to a $D_2O$ solution of β-CD, the spectra show marked changes in chemical shift of proton resonances of H-3 and H-5 of β-CD, which are located in the interior of the cavity,[11] while the resonances of H-1, H-2, H-4, and H-6, which are located in the exterior of the cavity,[11] remain unchanged. The results reveal that Dimer **2** is included in the interior of the β-CD cavity to form an inclusion complex. The $^1$H NMR spectra of α-CD and γ-CD in the absence and the presence of Dimer **2** were recorded too. Fig. 4 shows a comparison of changes in chemical shift of protons of α-, β-, and γ-CD upon addition of Dimer **2** in $D_2O$ solution. Only H-3 and H-5 of β-CD shift markedly upon complexation with Dimer **2**. For all protons of α-CD and γ-CD, no marked changes in chemical shift were observed, because Dimer **2** does not form inclusion complexes with α-CD or γ-CD.

The stoichiometry of the inclusion complex formation between Dimer **2** and β-CD may be obtained by continuous variation experiments, *i.e.*, recording $^1$H NMR spectra for solutions where the molar ratio of Dimer **2** and β-CD is varied but the overall molar concentration of the two components is kept constant. Fig. 5 shows the continuous variation plots (Job plots[14]) for changes in chemical shift of protons of Dimer **2** (a) and β-CD (b). Plots for all protons in Fig. 5 show a maximum at a molar fraction of Dimer **2** or β-CD equal to 0.5, indicating

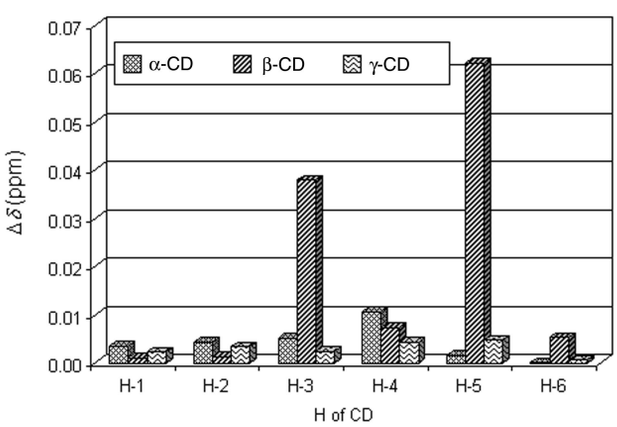


**Fig. 4** Changes in $^1$H NMR chemical shift for protons of α-, β-, and γ-CD induced by Dimer **2** in $D_2O$ at 27 °C. The concentration for Dimer **2**–CD is 15.0 : 5.0 mmol dm$^{-3}$.

the formation of a 1 : 1 inclusion complex. The stoichiometry is the same as for the complex formation between Dimer **1** and β-CD as we previously reported.[13]

### Dissociation constant and thermodynamic parameters

Since Dimer **2** and β-CD form a 1 : 1 inclusion complex, the equilibrium of the complex may be expressed by:

$$D + CD \underset{K_d}{\overset{K_a}{\rightleftharpoons}} D{\cdot}CD \quad (1)$$

Then, the dissociation constant $K_d$ for the complex formation is given by:

$$K_d = [D][CD]/[D{\cdot}CD] \quad (2)$$

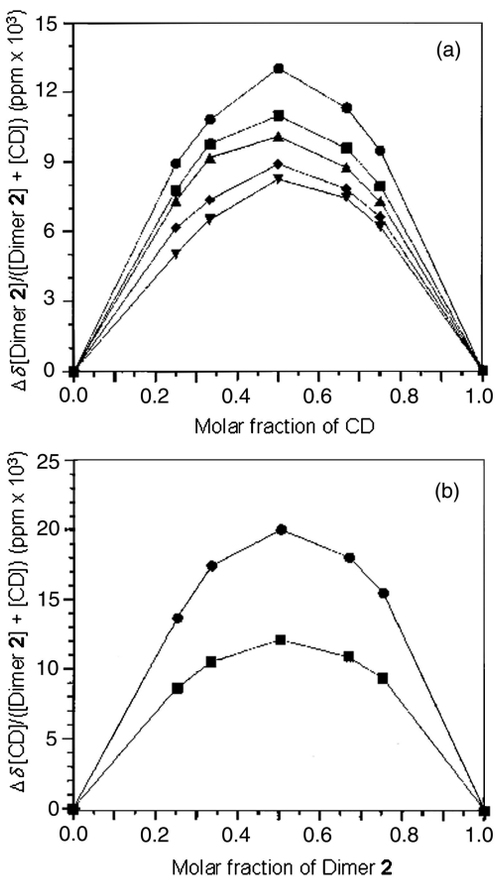


**Fig. 5** Continuous variation plots for Dimer **2**–β-CD system in $D_2O$ at 27 °C. (a) Job plots for changes in chemical shift of H3 (●), $-OCH_3$ (■), H4 (▲), H2 (◆), and H3′ (▼) of Dimer **2**; (b) Job plots for changes in chemical shift of H-5 (●) and H-3 (■) of β-CD.

where [D] and [CD] are the equilibrium concentrations of Dimer **2** and β-CD, respectively, and [D·CD] is the equilibrium concentration of the complex. For a proton of Dimer **2**, the observed chemical shift $\delta = \delta_D + ([D{\cdot}CD]/[D]_0)(\delta_{D{\cdot}CD} - \delta_D)$, where $[D]_0$ is the initial concentration of Dimer **2**, and $\delta_D$ and $\delta_{D{\cdot}CD}$ are the chemical shifts of the proton in pure Dimer **2** and in pure complex, respectively. Therefore, the observed induced chemical shift $\Delta\delta$ is:

$$\Delta\delta = \delta - \delta_D = ([D{\cdot}CD]/[D]_0)\Delta\delta_{D{\cdot}CD} \quad (3)$$

where $\Delta\delta_{D{\cdot}CD} = \delta_{D{\cdot}CD} - \delta_D$. The following equation then is applicable from eqns. (2) and (3):

$$1/\Delta\delta = 1/\Delta\delta_{D{\cdot}CD} + (K_d/\Delta\delta_{D{\cdot}CD})(1/[CD]) \quad (4)$$

If one is working under a condition of initial β-CD concentration $[CD]_0$ in large excess over $[D]_0$, then eqn. (4) becomes:

$$1/\Delta\delta = 1/\Delta\delta_{D{\cdot}CD} + (K_d/\Delta\delta_{D{\cdot}CD})(1/[CD]_0) \quad (5)$$

This is an analogue of the Benesi–Hildebrand equation.[15] For a series of solutions, a plot of $1/\Delta\delta$ against $1/[CD]_0$ should be linear. The intercept with the ordinate yields $1/\Delta\delta_{D{\cdot}CD}$ and from the gradient $K_d/\Delta\delta_{D{\cdot}CD}$ may be obtained.

However, our conditions were only $[CD]_0 > 5$. So we first obtained approximate values of $\Delta\delta_{D{\cdot}CD}$ and $K_d$ by using eqn. (5). The values were taken to calculate [CD] in the given solutions. Then we estimated new values of $\Delta\delta_{D{\cdot}CD}$ and $K_d$ by using eqn. (4). These procedures were repeated until new values of $\Delta\delta_{D{\cdot}CD}$ and $K_d$ were identical to previous ones.[16] For our data, the identical values were reached after three repetitions. The dissociation constants $K_d$ at various temperatures between 17 and 45 °C were determined. The temperature dependence of the dissociation constants yields an enthalpy $\Delta H°$ of −6.5 kcal $mol^{-1}$ for the complex formation. The free energy $\Delta G°$ and the entropy $\Delta S°$ were also calculated. Table 1 summarizes all of the thermodynamic parameters for the complex formation between Dimer **2** and β-CD. For comparison, thermodynamic parameters for complex formation between Dimer **1** and β-CD are also listed in the table. It is clear that the driving force for formation of both complexes is the large favorable enthalpy. It seems that the large favorable enthalpy is due to the contribution of van der Waals forces and hydrogen bonding between the host and the guest molecules, while the unfavorable entropy is due to the loss of conformational and rotational freedom of the dimers and/or conformational freedom of β-CD.[17] The changes both in enthalpy and in entropy reveal a tight inclusion and a deep penetration of the guest molecules within the cavity of β-CD. A key factor which affects the complex formation is the size correspondence between the dimers and the β-CD cavity. Therefore, we can understand why the 3HB dimers do not form complexes with α-CD and γ-CD, whose cavities are too small or too large for the 3HB dimers. Another factor is the capability to form hydrogen bonding between the 3HB dimers and β-CD. The hydroxy end group of both Dimer **1** and Dimer **2** can form hydrogen bonding with β-CD. However, Dimer **1** has another free carboxy end group capable of forming hydrogen bonding with β-CD more efficiently than Dimer **2**, whose corresponding end group is a methyl ester. This may be a primary reason that the enthalpy for Dimer **1**–β-CD is larger than that for Dimer **2**–β-CD. The decrease in entropy for Dimer **1**–β-CD is larger than that for Dimer **2**–β-CD, which may result from loss of more rotational freedom of Dimer **1** due to formation of more hydrogen bonding between the guest molecule and β-CD.

## Conformational analysis and structures of complexes

For each monomer unit of a 3HB dimer, there are three possible conformers: *trans* (T), *plus gauche* (G), and *minus gauche* ($\bar{G}$), as shown in Fig. 6. The two monomer units of a 3HB dimer show

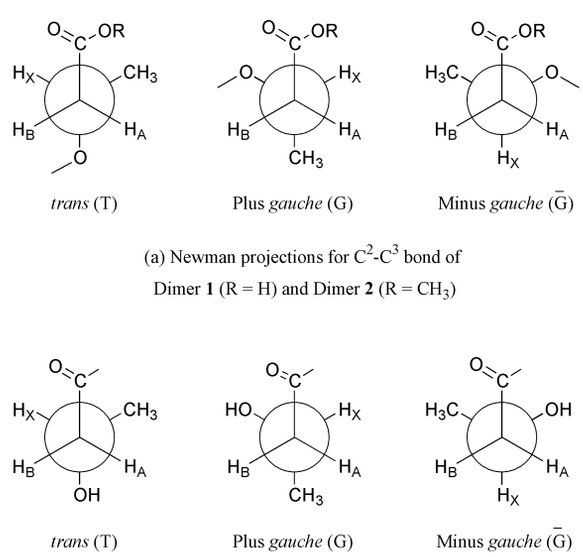


**Fig. 6** Newman projections of possible conformers of 3HB dimers for $C^2$–$C^3$ bond (a) and $C^{2'}$–$C^{3'}$ bond (b).

different conformational behavior due to formation of intramolecular hydrogen bonding and the different polarities of the solvents.[10] It is of great interest to analyze the conformations of the 3HB dimers in the complexes and make a comparison

**Table 1** Thermodynamic parameters for complex formation of 3HB dimers with β-CD in $D_2O$ solution at various temperatures[a]

| System | Temperature/°C | $K_d$/mol dm$^{-3}$ | $\Delta G°$/kcal mol$^{-1}$ | $\Delta H°$/kcal mol$^{-1}$ | $\Delta S°$/cal mol$^{-1}$ K$^{-1}$ |
|---|---|---|---|---|---|
| Dimer **2**–β-CD[b] | 17.0 | $1.07 \times 10^{-2}$ | −2.61 | −6.5 | −13.0 |
| | 27.0 | $1.58 \times 10^{-2}$ | −2.46 | | |
| | 35.0 | $2.49 \times 10^{-2}$ | −2.25 | | |
| | 45.0 | $3.27 \times 10^{-2}$ | −2.15 | | |
| Dimer **1**–β-CD[c] | 15.0 | $6.59 \times 10^{-3}$ | −2.87 | −10.4 | −25.6 |
| | 27.0 | $1.06 \times 10^{-2}$ | −2.71 | | |
| | 37.0 | $2.56 \times 10^{-2}$ | −2.21 | | |
| | 47.0 | $3.69 \times 10^{-2}$ | −2.10 | | |

[a] Parameters were determined from $^1$H NMR titration. [b] This work. Chemical shift changes of H4 were used for the calculations. [c] Data from ref. 13.

**Table 2** Coupling constants of methylene protons (H2 and H2′) in 500-MHz $^1$H NMR spectra and conformer distributions of $CH_2$–CH bonds of 3HB dimers in $D_2O$ solution in the absence and the presence of β-CD at 27 °C

| | | | Coupling constant/Hz | | Conformer fraction | | |
|---|---|---|---|---|---|---|---|
| System | Bond | Probe H | $J_{AX}$ | $J_{BX}$ | $P_T$ | $P_G$ | $P_{\bar{G}}$ |
| Dimer **1**[a] | $C^2$–$C^3$ | H2 | 6.6 | 6.6 | 0.50 | 0.50 | 0.00 |
| | $C^{2'}$–$C^{3'}$ | H2′ | 5.5 | 7.3 | 0.38 | 0.58 | 0.04 |
| Dimer **1**–β-CD[b] | $C^2$–$C^3$ | H2 | 7.4 | 6.0 | 0.58 | 0.43 | 0.00 |
| | $C^{2'}$–$C^{3'}$ | H2′ | 7.4 | 6.0 | 0.58 | 0.43 | 0.00 |
| Dimer **2**[c] | $C^2$–$C^3$ | H2 | 6.4 | 6.4 | 0.49 | 0.49 | 0.02 |
| | $C^{2'}$–$C^{3'}$ | H2′ | 5.2 | 7.8 | 0.35 | 0.64 | 0.00 |
| Dimer **2**–β-CD[d] | $C^2$–$C^3$ | H2 | 7.3 | 6.1 | 0.57 | 0.44 | 0.00 |
| | $C^{2'}$–$C^{3'}$ | H2′ | 7.3 | 5.8 | 0.57 | 0.41 | 0.02 |

[a] [Dimer **1**] = 5.0 mmol dm$^{-3}$. [b] [Dimer **1**] = 5.0 mmol dm$^{-3}$; [β-CD] = 15.0 mmol dm$^{-3}$. [c] [Dimer **2**] = 5.0 mmol dm$^{-3}$. [d] [Dimer **2**] = 5.0 mmol dm$^{-3}$; [β-CD] = 15.0 mmol dm$^{-3}$.

of the conformational structures between the complexed and uncomplexed dimers.

As described in previous papers,[10,18] the distribution of conformers around the $CH_2$–CH bonds of a 3HB unit in solution can be determined by means of $^1$H NMR spectroscopy. The methylene proton resonances are associated with the methine proton ($H_X$) and are analyzed as an ABX three-spin system with a vicinal coupling of $H_A$ and $H_B$ protons. Then, the coupling constants $J_{AX}$ and $J_{BX}$ are presented by average values of the component coupling constants in the three conformers weighted by their fractional populations $P_T$, $P_G$, and $P_{\bar{G}}$, as follows:

$$J_{AX} = P_T J_t + P_G J_g + P_{\bar{G}} J_g \quad (6)$$

$$J_{BX} = P_T J_g + P_G J_t + P_{\bar{G}} J_g \quad (7)$$

$$1 = P_T + P_G + P_{\bar{G}} \quad (8)$$

where $J_g$ and $J_t$ are the *gauche* and *trans* vicinal coupling constants, respectively. Assuming the reasonable values of $J_g = 2.1$ Hz and $J_t = 11.0$ Hz,[19] we can calculate the fractional populations $P_T$, $P_G$, and $P_{\bar{G}}$ for the $CH_2$–CH bonds under various conditions.

Table 2 summarizes the coupling constants of methylene protons and conformer distributions of $CH_2$–CH bonds in 3HB dimers in $D_2O$ solution obtained from the $^1$H NMR spectra of 3HB dimers in solution and in β-CD complexes. For the $C^2$–$C^3$ bond of both free 3HB dimers in $D_2O$ solution, predominant conformers are *trans* and *plus gauche*, while the *minus gauche* conformer is strongly disfavored. This is in accordance with the steric energies of the *trans* and *plus gauche* conformers being much lower than that of the *minus gauche* conformer for both 3HB dimers, because the carbonyl oxygen receives van der Waals repulsion from only one of either $–CH_3$ or –OR groups in the *trans* and *plus gauche* conformers, but from both $–CH_3$ and –OR groups in the *minus gauche* conformer. For the $C^2$–$C^3$ bond of both 3HB dimers in β-CD complexes, the most preferred conformer becomes *trans*, while the *plus gauche* conformer becomes the next preferred. The conformational distribution is more like the case in a non-polar organic solvent.[10] The results indicate that the complexed 3HB dimers are in a different environment from that formed by $D_2O$ solvent, where the dimers have less chance to interact with the aqueous molecules.

For the $C^{2'}$–$C^{3'}$ bond of both free 3HB dimers in $D_2O$ solution, the predominant conformer is *plus gauche*, and the next preferred conformer is *trans*, while the *minus gauche* conformer is strongly disfavored (Table 2). As described in our previous papers,[10] the *plus gauche* conformer of the $C^{2'}$–$C^{3'}$ bond of 3HB dimers in $D_2O$ solution is stabilized by the formation of an intramolecular hydrogen bond between the hydroxy end group and the vicinal carbonyl group. However, for the $C^{2'}$–$C^{3'}$ bond of both 3HB dimers in β-CD complexes, the most preferred conformer becomes *trans*, while the *plus gauche* conformer becomes the next preferred, indicating that the intramolecular hydrogen bond is broken. It seems that the hydroxy end group of the 3HB dimers tends to form hydrogen bonding with β-CD, which contributes to the favorable enthalpy for the complexation, as discussed in a previous section of this paper.

Table 3 summarizes $^1$H NMR chemical shifts of Dimer **2** in the absence and the presence of β-CD and the chemical shifts for protons of Dimer **2** induced by β-CD in $D_2O$. The induced chemical shifts for H2, H3, and H4 are more marked than those for H2′, H3′, and H4′, respectively, indicating that the 3HB unit with a $–COOCH_3$ end group is more subject to the effect of the inner wall of the cavity of β-CD. A probable explanation of the results is that the 3HB unit with a $–COOCH_3$ end group is included in the narrow side of the β-CD cavity. Such geometry makes the 3HB unit with a $–COOCH_3$ end group closer to the inner wall of the β-CD cavity, and the chemical shifts of protons of the 3HB unit with a $–COOCH_3$ end group are more subject to the effect of β-CD. Based on the data of $^1$H NMR induced chemical shifts, and the thermodynamic and conformational analyses, a schematic illustration of a proposed structure of the Dimer **2**–β-CD complex is shown in Fig. 7. In the complex, the hydroxy end of Dimer **2** forms

**Table 3** $^1$H NMR chemical shifts of Dimer **2** in the absence and the presence of β-CD and the chemical shifts of Dimer **2** ($\Delta\delta$) induced by β-CD in $D_2O$ at 27 °C

| | Chemical shift of protons of Dimer **2** (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | $-OCH_3$ | H2 | H2′ | H3 | H3′ | H4 | H4′ |
| Dimer **2**[a] | 3.690 | 2.717 | 2.507 | 5.281 | 4.196 | 1.299 | 1.209 |
| Dimer **2**–β-CD[b] | 3.722 | 2.691 | 2.495 | 5.243 | 4.172 | 1.328 | 1.217 |
| $\Delta\delta$ | 0.032 | −0.026 | −0.012 | −0.038 | −0.025 | 0.029 | 0.008 |

[a] [Dimer **2**] = 5.0 mmol dm$^{-3}$. [b] [Dimer **2**] = 5.0 mmol dm$^{-3}$; [β-CD] = 15.0 mmol dm$^{-3}$.

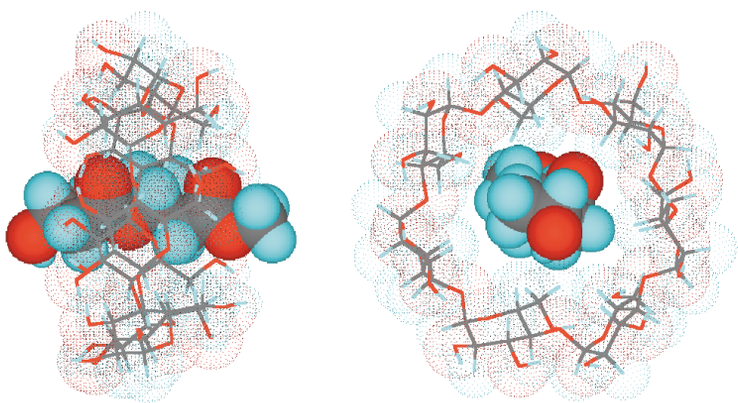

**Fig. 7** Proposed structure of Dimer **2**–β-CD complex (left, side view; right, top view).

hydrogen bonding with one of the secondary hydroxy groups of β-CD.

## Conclusions

Two 3HB dimers, Dimer **1** and Dimer **2**, selectively form inclusion complexes with β-CD in $D_2O$ solution, while 3HB monomer does not form any complex with cyclodextrins. The thermodynamic analysis reveals that the complex formation is enthalpically favorable but entropically unfavorable, indicating that the driving forces for the complex formation are van der Waals forces and hydrogen bonding. A comparison of the thermodynamic parameters for complex formation of the two 3HB dimers with β-CD reveals that Dimer **1** may form hydrogen bonding more efficiently than Dimer **2**, while the decrease in entropy for Dimer **1**–β-CD is larger than that for Dimer **2**–β-CD, which may result from loss of more rotational freedom of Dimer **1** due to formation of more hydrogen bonding between the guest molecule and β-CD. Both the selectivity and the thermodynamic analysis imply that the size correspondence between the guest and host molecules plays an important role in the complex formation.

Conformational analysis of the 3HB dimers in solution and in the complexes indicates that both $C^2$–$C^3$ and $C^{2'}$–$C^{3'}$ bonds of the two 3HB dimers in β-CD complexes mainly take *trans* conformations, which is different from the case of the free 3HB dimers in $D_2O$ solution. A 3HB dimer complexed in the cavity of β-CD is in a different environment from that formed by $D_2O$ solvent, where the dimer has less chance to interact with the solvent molecules. In $D_2O$ solution, an intramolecular hydrogen bond between the hydroxy end group and the oxygen of the adjacent carbonyl group is formed and the $C^{2'}$–$C^{3'}$ bond takes mainly the *plus gauche* conformation for the two 3HB dimers. In the complexes, instead of the intramolecular hydrogen bonding, the hydroxy end group forms hydrogen bonding with β-CD, and the $C^{2'}$–$C^{3'}$ bond takes mainly the *trans* conformation for the two 3HB dimers. It is also concluded that the 3HB unit with a $-COOCH_3$ or –COOH end group is included in the narrow side of the β-CD cavity from an analysis of the $^1$H NMR induced chemical shifts for respective protons of the 3HB dimers.

## Experimental

### Materials

α-CD, β-CD, and γ-CD were purchased from Wako Pure Chemical Ind. Ltd. Other chemicals for synthesis were supplied by Aldrich. Deuterium oxide ($D_2O$, 99.95%) was obtained from Merck. Dimer **1** and Dimer **2** were prepared according to the procedures described in our previous papers.[10]

Dimer **1**: $^1$H NMR $\delta_H$(500 MHz; $CDCl_3$; DSS) 5.34 (m, 1H, C(3)H), 4.21 (m, 1H, C(3′)H), 2.55–2.69 (m, 2H, C(2)$H_2$), 2.39–2.48 (m, 2H, C(2′)$H_2$), 1.33 (d, $J$ = 6.4 Hz, 3H, C(4)$H_3$), 1.23 (d, $J$ = 6.4 Hz, 3H, C(4′)$H_3$). FAB-MS $m/z$ 191.3 ($M^+$ + l; 100%), 105.6 (57%). Elemental Anal. Found: C, 49.80; H, 7.44%. Calc. for $C_8H_{14}O_5$: C 50.52; H, 7.42%.

Dimer **2**: $^1$H NMR $\delta_H$(500 MHz; $CDCl_3$; DSS) 5.29–5.36 (m, 1H, C(3)H), 4.16–4.22 (m, 1H, C(3′)H), 3.69 (s, 3H, $OCH_3$), 3.05 (s, broad, 1H, OH), 2.51–2.67 (m, 2H, C(2)$H_2$), 2.37–2.48 (m, 2H, C(2′)$H_2$), 1.32 (d, $J$ = 6.1 Hz, 3H, C(4)$H_3$), 1.23 (d, $J$ = 6.4 Hz, 3H, C(4′)$H_3$). FAB-MS $m/z$ 205.0 ($M^+$ + 1; 100%), 119.2 (44%). Elemental Anal. Found: C, 50.83; H, 7.68%. Calc. for $C_9H_{16}O_5 \cdot 0.4H_2O$: C, 51.13; H, 8.01%.

### Measurements

The $^1$H and $^{13}$C NMR spectra were recorded on a JEOL GX-500 NMR spectrometer at 500 and 125 MHz, respectively. The $^1$H NMR measurements were carried out with 5.3-s pulse repetition, 5000-Hz spectral width, and 32768 data points. Chemical shifts were referred to DSS ($\delta$ = 0.00 ppm) as external reference in a $D_2O$ solution. Mass spectra were obtained on a JEOL JMS-HX 110 mass spectrometer by the fast atom bombardment (FAB) method in positive ion mode with a glycerol matrix, 5.0–10.0 kV acceleration voltage, and 1000 resolution. Mass calibration was carried out with a CsI spectrum.

### Continuous variation experiments

The total concentrations of 3HB dimer and β-CD were maintained at 20.0 mmol dm$^{-3}$ in $D_2O$, while the molar ratios of 3HB dimer and β-CD were varied to be 1 : 0, 3 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3, and 0 : 1. The $^1$H NMR spectra of the samples were

recorded at 27 °C. The Job plots[14] were drawn based on the induced chemical shifts of protons of 3HB dimer and β-CD, respectively.

### Determination of dissociation constant

A series of $D_2O$ solutions which include 1.5 mmol $dm^{-3}$ of 3HB dimer and 0.0, 8.0, 10.0, 12.0, 14.0, and 16.0 mmol $dm^{-3}$ of β-CD were prepared. The $^1H$ NMR spectra of the solutions were recorded at different temperatures. The dissociation constants and other thermodynamic parameters were estimated from the induced chemical shifts of H4 of Dimer **2**. The average chemical shift values for the double peaks of H4 were used.

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